1979

Helix-Coil Transitions of Homopolypeptides Induced by Salts and Detergents.

Robert W. Mccord

Louisiana State University and Agricultural & Mechanical College

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BY SALTS AND DETERGENTS

The Louisiana State University and Agricultural
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HELIX-COIL TRANSITIONS OF HOMOPOLYPEPTIDES INDUCED

BY SALTS AND DETERGENTS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Biochemistry

by

Robert W. McCord
B.A., University of North Florida, 1974
December 1979
EXAMINATION AND ThESIS REPORT

Candidate: Robert W. McCord

Major Field: Biochemistry

Title of Thesis: HELIX-COIL TRANSITIONS OF HOMOPOLYPEPTIDES
INDUCED BY SALTS AND DETERGENTS

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EXAMINING COMMITTEE:

Date of Examination:

August 24, 1979
ACKNOWLEDGEMENT

This author would like to express his appreciation and respect for Dr. Wayne L. Mattice, without whose guidance, encouragement, leadership and support this work would not have been completed.

Aid in many forms from Drs. E. S. Younathan, S. A. Chang, E. A. Blakeney, G. E. Risinger, R. S. Allen and D. K. Carpenter has often been unacknowledged but always appreciated.

To Dr. Barbara E. Torgerson, friend and wife, thank you for your undying support. Penultimate thanks are offered to the minions of Mole Man—the Scowl, Wombat, Super Hack, Boney and Chuckles—for various support and aid.

The financial support of the LSU Biochemistry Department and National Science Foundation is gratefully acknowledged and appreciated.
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ABSTRACT

The uncharged polymers, PHEG [poly(N<sup>5</sup>-ω-hydroxyethyl-L-glutamine)], PHPG [poly(N<sup>5</sup>-ω-hydroxypropyl-L-glutamine)], PHBG [poly(N<sup>5</sup>-ω-hydroxybutyl-L-glutamine)] and the uncharged oligomer Ac(Ala)<sub>3</sub>OMe (Acetyl-L-alanyl-L-alanyl-L-alanine methyl ester) have been used to investigate salt effects on helix-coil transitions. The measurement of the ellipticity at 222 nm was used to calculate fraction helix. The ellipticity at 222 nm of the completely helical peptide was assumed from the ellipticity of poly-L-glutamic acid at low pH and low temperature. The ellipticity at 222 nm of the completely coil peptide was estimated from that of Ac(Ala)<sub>3</sub>OMe. The salts employed were NaClO<sub>4</sub> and CaCl<sub>2</sub>. PHEG is a random coil in water, whereas PHBG is mostly helix at low temperatures. PHPG has intermediate helix content ranging from 23% to 2% in water from 5°C to 70°C, respectively. Circular dichroism was used to determine fraction helix. At less than 2 M concentration NaClO<sub>4</sub> helix formation was induced in all three polymers at all temperatures investigated. At higher concentrations the helix was destabilized. CaCl<sub>2</sub> destabilizes the α-helix at low activities. At high activities the α-helix is stabilized and at activities above 30 m, measurable α-helix is observed. The ellipticity of Ac(Ala)<sub>3</sub>OMe provided the
estimation of ellipticity of the random coil at various temperatures and salt concentrations.

The effects of SDS (sodium dodecyl sulfate) on the charged polymer, poly-L-histidine, and DA (dodecylamine) on poly-L-glutamic acid were investigated. The detergents caused coil to helix and coil to ordered structure transitions for poly-L-glutamic acid and poly-L-histidine, respectively. These transitions occurred at detergent to residue ratios near one.

The salt effect data were analyzed using a curvefitting program employing equations generated from a Zimm-Bragg treatment of helix-coil transitions. Standard Zimm-Bragg analysis of the data could not produce a reasonable fit of the data. Cooperativity of binding was invoked and provided excellent agreement with the data and a plausible explanation of the salt effects observed. The binding of the salts to the coil proved to be non-cooperative, whereas binding of NaClO₄ to the helix proved to be anticooperative and the binding of CaCl₂ to the helix, cooperative.

By using the modified equation, binding constants of the salts to the helix and coil residues were calculated for PHEG, PHPG, and PHBG. The thermodynamic functions ΔH, ΔS, and ΔG for the binding of each salt to the coil and helix were calculated from the binding constants at various temperatures. The values of ΔH° and ΔS°, calculated for NaClO₄, agreed with reported values for the binding of SCN⁻ to Methemoglobin A and showed the phenomenon of enthalpy-entropy compensation.
A model for the binding of the salts to the helix surface is suggested. This model represents the helix surface as a lattice to which salts may bind. Interactions of the salt with more than one point on the lattice leads to cooperativity. The amide dipoles in the R-groups of these polymers provide a binding site which can interact with other binding sites.
Chapter I

INTRODUCTION

Proteins have numerous functions in the living organism. Many of these functions are readily apparent, such as the structural role played by collagen of connective tissue or keratin of hair. Other protein functions are more subtle, for example, the fine control of metabolism by enzyme, carrier, storage, regulatory, hormone, and transport proteins, or the role of immunoglobulins in mammalian disease-defense systems.

The one unifying factor of protein function is conformation. Each of the proteins mentioned above will lose its function if its conformation is significantly changed, as by denaturation. Increasing the temperature of a protein's environment within the temperature range of liquid water is sufficient to denature most known proteins. Changes in the salt concentration or hydrophobicity of the solvent are capable of denaturing proteins. In the milieu of the living organism proteins are constantly faced with perturbations of environment. Perturbations can be quite drastic, as when pepsinogen is secreted into the stomach lumen, or rather subtle, as the effect of minute concentrations of citrate on the activity of phospho-fructokinase (1). The high correlation of protein environment with conformation and in turn function is indication for a study of environmental factors that affect protein structure.
The structure of proteins has been divided into primary, secondary, tertiary, quarternary and recently pentiary classes. Primary structure deals with the amino acid makeup of proteins. Proteins are composed of amino acid units linked together by amide bonds between consecutive α-amino and carboxyl groups. The primary structure is then the sequence of amino acid residues of a protein listed by convention from the amino to carboxy terminus. The recurring, regular arrangement of adjacent peptide residues in space is called secondary structure. In this paper secondary structure will also include nonregular portions of a globular protein that recur in molecule after molecule of that protein. Such structures have, unfortunately, been labeled "random" by crystallographers, but are not the random coil conformation which will be discussed in greater detail in this paper. Regular secondary structures are helices and the so-called "pleated sheet." Tertiary structure refers to the folding of secondary structures into compact globular proteins. The arrangement of the individual chains of a protein with more than one noncovalently linked peptide chain is termed quarternary structure. Pentiary structure refers to the arrangement of proteins in membranes or other complicated structures such as enzyme complexes.

Fig. 1 shows the configuration of a section of peptide chain. The bond lengths and angles are given in the figure. Conformation of the peptide backbone is determined by a set of angles $\phi_i$, $\psi_i$, and $\omega_i$ which are defined as the dihedral angles about $N_i-C_i^\alpha$, $C_i^\alpha-C_i'$, and the peptide bond, $i$, respectively. By convention these angles are
Figure 1. Geometrical representation of a portion of a polypeptide chain (166).
measured as clockwise rotations about the indicated bond when viewed from the amino to carboxy terminus, with zero defined as follows:

a. $\phi_i = 0$ when $C'_{i-1}$ and $C'_{i}$ are trans.

b. $\psi_i = 0$ when $N_i$ and $N_{i+1}$ are trans.

c. $\omega_i = 0$ when $C^\alpha_{i}$ and $C^\alpha_{i+1}$ are trans.

The convention for determining the zero reference angles for $\phi$, $\psi$ and $\omega$ are those of the IUPAC-IUB Commission on Biochemical Nomenclature of 1966 (2). In 1969 the commission changed the designation of these angles to better agree with current organic chemical nomenclature (3). The new designation of $\phi$, $\psi$, and $\omega$ can be derived from the 1966 convention by subtracting $180^\circ$ from each angle. The convention above is the 1966 nomenclature which will be used in this dissertation because it is convenient in protein conformational studies to assume the completely extended chain to be in the reference conformation.

The peptide bond is planar trans, and thus $\omega = 0^\circ$ for almost all cases. Prolyl residues provide an exception to the previous statement in that the cis conformation about the peptide bond is common (4,24). If $\omega = 0^\circ$ there remain only two orders of freedom, $\phi$ and $\psi$, for each residue that describe the peptide backbone conformation. Therefore, the entire conformation of the peptide chain can be specified by a set of angles, $\phi_i$, $\psi_i$. If all $\phi_i$, $\psi_i$ are equal then the polypeptide is defined as a helix.

Various helices can be specified by $\phi$, $\psi$ angles or by a set of parameters $n$, $d$, and $p$ which represent respectively the number of
residues per complete turn, the distance along the helical axis required for one turn, called the rise, and the product of n and d, called the pitch. (See Table 1.) The helix also has the quality of sense with a designation of either right or left handed. A right-handed helix is one that when viewed from either end spirals away in a clockwise fashion. The three types of helices commonly found in native proteins are the α-helix, the collagen triple helix and the β-pleated sheet. The β-structure is often considered apart from other helices because of the sheet like, side-by-side arrangement of a variable number of chains necessary to form both interchain and intrachain hydrogen bonds that give this structure its stability.

The polypeptide chains (helices) of β-structure can be parallel or antiparallel, depending on whether the chains run in the same direction or alternate direction. Homopolypeptides in which the γ atom of the residue is oxygen or sulfur, such as poly-L-serine, poly-L-cysteine, and poly-L-threonine, tend to form the pleated sheet. It is believed that dipole-dipole interactions of adjacent groups destabilize the α-helix, but such interactions are minimized in the β-structure. Nature's best example of the pleated sheet is silk fibroin from Bombyx mori which contains 12-16% serine (5,6). Poly-L-lysine and poly-L-histidine have been shown to form β-structures under proper solvent conditions (7,8). Some native globular proteins have various amounts of β-structure as indicated by x-ray crystallography. (See Table 2.)
Table 1. Parameters of ordered secondary protein structures. Parameters in parentheses for α-helix from (129) and 3_{10} from (130). All others from (31).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>α-Helix</th>
<th>3_{10} Helix</th>
<th>2_7 Ribbon</th>
<th>Polyproline helix</th>
<th>Antiparallel β-pleated sheet</th>
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<tr>
<td>( \phi )</td>
<td>132°(113°)</td>
<td>131°(106°)</td>
<td>105°</td>
<td>103°</td>
<td>40°</td>
</tr>
<tr>
<td>( \psi )</td>
<td>123°(136°)</td>
<td>154°(176°)</td>
<td>250°</td>
<td>326°</td>
<td>315°</td>
</tr>
<tr>
<td>( \eta )</td>
<td>3.61</td>
<td>3.00</td>
<td>2.00</td>
<td>-3.00*</td>
<td>2.0</td>
</tr>
<tr>
<td>( d(Å) )</td>
<td>1.5</td>
<td>2.0</td>
<td>2.80</td>
<td>3.12</td>
<td>3.47</td>
</tr>
<tr>
<td>( p(Å) )</td>
<td>5.41</td>
<td>6.0</td>
<td>5.60</td>
<td>-9.36</td>
<td>6.95</td>
</tr>
</tbody>
</table>

*The negative sign denotes a left-handed helix.
Alpha helices are found in nature in both structural and globular proteins. The $\phi$ and $\psi$ angles and the rise, pitch, and residues per turn are listed in Table 1. The table indicates that there is a small range of $\phi$ and $\psi$ that yield the $\alpha$-helix. The $\alpha$-helix is not only found in nature, it is also the conformation adopted by many homopolypeptides in a variety of solvents. These helices have been right- or left-handed with the sense, a function of the side chain. Homopolymers of L-alanine, L-lysine, L-tyrosine, $\beta$-propyl-L-aspartate, $\gamma$-benzyl-L-glutamate, $\gamma$-methyl-L-glutamate, $\epsilon$-benzyloxy-carbonyl-L-lysine, L-glutamic acid and $N^5$(4-hydroxybutyl-L-glutamine) have been demonstrated to be right-handed helices in non-interacting solvents (9-17). On the other hand, $\beta$-methyl-, $\beta$-benzyl-, $\beta$-($o$-chlorobenzyl)-, and $\beta$-($m$-chlorobenzyl)-L-aspartate have demonstrated formation of the left-handed $\alpha$-helix (9-17).

The naturally-occurring structural proteins $\alpha$-keratin of hair (22) and myosin of muscles (19,20) are examples of $\alpha$-helices. Both proteins are found in nature in superstructures. $\alpha$-keratin is believed to consist of three $\alpha$-helices slightly wound about each other while parts of myosin consist of two entwined $\alpha$-helices (21). Globular proteins contain varying amounts of $\alpha$-helices. Concanavalin A has no $\alpha$-helix (23), whereas others, for example myoglobin (Table 2) have greater amounts of $\alpha$-helix. Table 2 gives the approximate contribution of $\alpha$-helix and $\beta$-structure to the tertiary structure of some globular proteins.
Table 2. Secondary structure contribution to the tertiary structure of selected proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Reference</th>
<th>Helix*</th>
<th>β-Sheet</th>
<th>Nonregular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>(127)</td>
<td>27%</td>
<td>15%</td>
<td>58%</td>
</tr>
<tr>
<td>Ribonuclease S</td>
<td>(127)</td>
<td>23%</td>
<td>43%</td>
<td>34%</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>(127)</td>
<td>79%</td>
<td>0%</td>
<td>21%</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>(128)</td>
<td>0%</td>
<td>57%</td>
<td>43%</td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
<td>(127)</td>
<td>20%</td>
<td>35%</td>
<td>45%</td>
</tr>
</tbody>
</table>

* Small, non-α-helical, helical segments are included in this calculated value, e.g. residue 80-85 in lysozyme is intermediate between the α and 3_{10} helices (127).
Figure 2. Hydrogen bonding pattern of the alpha-helix.
Figure 2. Hydrogen bonding pattern of the α-helix.
The collagen helix is a slightly twisted triple helix in which two of every three amino acid residues are involved in inter-chain hydrogen bonds (25). The individual chains are left-handed helices, but are wrapped around each other with a slight right-handed twist. The conformation of this structure demands that every third residue be glycyl. Close packing requirements of the chains as they twist around each other provide no room for a side chain at every third residue. There are known helical peptide structures, similar to collagen, which have not been isolated from living systems. Such helical structures include those for the homopolypeptides polyglycine II (26), poly-L-proline (form II) (27-29) and poly-L-hydroxyproline A (30), all of which adopt helices similar to the left-handed helix of collagen. Polyglycine, however, can just as easily adopt a right-as a left-handed helix due to the symmetry of the glycyl residue. Other possible helical structures are the π helix, $3_{10}$ helix, and poly-L-proline (form I) structure. Parameters for these helices are listed in Table 1.

The stability of helical structures lies partially in their ability to form interchain and intrachain hydrogen bonds. An alternate naming system names the helix according to the number of atoms in the chain connecting the hydrogen and the oxygen of a hydrogen bond and the fraction of residues involved in that bond. Examples of this type of nomenclature are the $2_7$ and $3_{10}$ helices. The α-helix would be the $3.7_{13}$ helix since there are 13 atoms and 3.7 residues per turn. (See Figure 2.)
If hydrogen bond formation is the driving force toward helix formation, why, with the numerous possible intrachain hydrogen bonding schemes, are so few types of helix observed? The answer to this question lies partially in steric interactions of the peptide atom groups as $\phi$ and $\psi$ are varied over 360 degrees. G. N. Ramachandran headed a group in India that used models and computers to calculate, over the range of $\phi$ and $\psi$, what conformations are impossible due to steric hindrance and what conformations are allowable for the L-alanyl residue (31). Using a hard sphere atom model, the group obtained a map of allowed and disallowed $\phi$, $\psi$ values. With a slight decrease in atomic radii a new area on the map was calculated. This area was termed the partially allowed area. Figure 3 shows the Ramachandran plot. The location of various helices is indicated on the map. These helices generally fall in or near allowed or partially allowed areas. Through a simple model Ramachandran was able to relate atomic to secondary structure of proteins, yet his work was obviously not the complete story. A more rigorous treatment of the same problem was performed by Paul Flory (32). He used a more refined model with more atomic interactions to produce energy maps for particular residues. These maps have a continuum of energy values instead of the three categories of Ramachandran's map. The conformational energies of a residue as a function of $\phi$, $\psi$ were calculated according to the following equation:
Figure 3. Ramachandran steric map (31) for the L-alanyl residue. "Normally allowed" regions are enclosed by solid lines; those meeting the requirements set by the "outer limit" distances are enclosed by dashed lines. Conformations $\phi,\psi$ corresponding to several helices are indicated as follows: right- and left-handed $\alpha$ helices, $\bigcirc$, $\pm 122^\circ$, $\pm 133^\circ$; parallel pleated sheet, $\boxplus$, $+62^\circ$, $-68^\circ$; anti-parallel pleated sheet, $\boxplus$, $38^\circ$, $-35^\circ$; polyglycine-II (left-handed) and poly-L-proline-II, $\square$, $102^\circ$, $-35^\circ$; collagen, $\ominus$, $123^\circ$, $-40^\circ$
\[ E(\phi,\psi) = \frac{E_0^\phi}{2}(1-\cos 3\phi) + \frac{E_0^\psi}{2}(1-\cos 3\psi) + \sum_k \sum_l E_{k,l}(\phi,\psi) + E_c \] Equation 1

\[ E_0^\phi \text{ and } E_0^\psi \text{ are the heights of the rotational barriers around the angles } \phi \text{ and } \psi. \] 

\[ E_0^\phi \text{ and } E_0^\psi \text{ are on the order of } 1.0 \text{ Kcal (33). The term } E_{k,l}(\phi,\psi) \text{ is defined as the energy of interaction of nonbonded atoms } k \text{ and } l \text{ separated by distance } r_{kl}, \text{ a function of } \phi \text{ and } \psi, \text{ and is the sum of the repulsive and attractive interactions as expressed in the following equation:} \]

\[ E_{k,l}(\phi,\psi) = a_{kl}/r_{kl}^m - c_{kl}/r_{kl}^6 \] Equation 2

The atomic pairs \( k,l \) are made up of nonbonded atoms from \( C_{i-1} \) to \( C_{i+1} \) whose separation distance \( r_{kl} \) is a function of \( \phi_k \) and \( \psi_l \). The exponent \( m \) is taken to be 12 (Leonard Jones potential). The attractive interaction \( c_{kl}/r_{kl}^6 \), where \( c_{kl} \) is a constant for atomic pair \( k,l \), is called the London dispersion interaction. The terms \( c_{kl} \) can be calculated from atomic polarizabilities using the Slater-Kirkwood equation (34). The terms \( a_{kl} \) are constants for the atomic pair \( k,l \), which can be arbitrarily chosen to reproduce observed bond distances in crystals. The \( a_{kl} \) were chosen to produce minima at the following atomic radii: \( r_H^O = 1.3 \text{Å} \), \( r_C^O = 1.8 \text{Å} \), \( r_C^H = 1.6 \text{Å} \), \( r_N^O = 1.65 \text{Å} \), and \( r_{CH_2}^O = 1.95 \text{Å} \) (32). \( E_c \) is the coulombic interaction between the adjoining pair of amide groups arising from the large dipole moment of the amide unit. This interaction destabilizes the \( \alpha \)-helix due to stacking of amide bonds.
The calculation of $E(\phi, \psi)$ yields energy maps that are similar to the Ramachandran work, but must be considered a refinement. Figures 4 and 5 show the energy plots for the glycyl and L-alanyl residues respectively. Specific information about the determination of $E_c$ is found in the legends. The various helices have been marked on the alanyl map for comparison with the Ramachandran plots. The presence of $-\text{H}$ as the R-group of the glycyl residue produces a symmetrical map. The results of the L-alanyl calculations are considered to be applicable to other residues with $-\text{CH}_2\text{R}$ side groups (35).

It is obvious that the ability of a polypeptide to form a helix is a function of the ability to form interchain or intrachain hydrogen bonds at an allowable $\phi, \psi$. However, there is more to helix formation than hydrogen bonding and steric considerations. For example, poly-L-glutamate does not form a helix in $\text{H}_2\text{O}$ at pH 7.6 (36,37), although it is not sterically hindered from forming any of several types of helices. The final determining factors of helix formation are side chain interactions and solvent interactions with the polypeptide. At pH 7.6 the carboxylate group of the side chain of poly-L-glutamate are charged and repulsion due to charge prevents the formation of the helix (38). The interactions of side chains and solvents will be further discussed later in this work. First a treatment of methods of describing and quantifying transitions to and from secondary structures is in order.
Figure 4. Conformational energy map (32) for the L-alanyl residue calculated according to Equation 1, with $E_c$ estimated on the basis of point monopole charges ($\varepsilon = 3.5$). The lowest minimum (X) occurs in region III.
Figure 5. Conformational energy map (32) for the glycy1 residue calculated according to Equation 1, with $E_c$ estimated on the basis of point monopole charges ($\epsilon = 3.5$). Contours are drawn at 1 kcal mole$^{-1}$ intervals.
There are two alternatives to ordered secondary structures. The first such alternative is one which is found in native globular proteins. X-ray crystallography has shown that parts of such proteins exist in nonregular chain segments. These segments have been called "random" segments. However, examination of all the molecules of the protein will likely show that most of the molecules have the same conformation of "random" segments. This recurring structure is designated nonregular secondary structure and the word random will not again be used in this work to refer to it. The second conformation alternative to the ordered secondary structure is a conformation that has no secondary structure at all. If a polypeptide is allowed to assume any values of $\phi$, $\psi$ according to constraints that arise from nearest neighbor interactions only, it will assume any one of a very large number of conformations in solution at any time. A group of chain molecules with no recurrent pattern of $\phi$, $\psi$ within or among the molecules, and with a statistical distribution of conformations, is called the random coil or coil in this paper. Statistical mechanics has been used to treat the conformation in which the molecule is free from inter- or intrachain long-range interactions and the effects of an external force. Such a conformation is said to exist in the unperturbed condition.

Proteins (39-41) and homopolypeptides (42-44) exist in random coil conformation in a variety of solvent conditions. Residues that exist in the random coil conformation have been postulated to exist even within crystalline proteins such as staphylococcal nuclease, which
has several residues that do not appear in the crystal structure as determined by x-ray scattering (45).

In native proteins three types of chain conformation can be demonstrated—ordered secondary, nonregular secondary and random coil conformation. By changing temperature, pressure, or solvent conditions, one is able to change the relative contribution of these conformations. This change can be visualized by movement on the map in Figure 6. The three extremes of this map are total nonregular structure, total ordered structure and total random coil. Most proteins in the native state would fall on the cross-hatched portion of the map. Perturbation by, for example, temperature change would move the protein toward the random coil corner. Different secondary structures of native protein have been shown to denature differently. Bovine α-lactalbumin denatures in high concentrations of NaClO₄ with preservation of the helical segments (46). Homopolypeptides are assumed to have no nonregular structure, so temperature change will move the peptide along the line between ordered structure and random coil. In special cases homopolypeptides will change from one ordered structure to another (α-helix to β-structure) (47). Such a change can not be represented by Figure 6. This dissertation deals mainly with transitions from ordered structure, namely the α-helix, to the random coil.

Theories employing statistical mechanics have been developed to explain helix-coil transitions. The first of these theories was that of Schellman (48) developed in 1955. This treatment employed an
Figure 6. A secondary structure map on which the following proteins and polypeptides are located: +, lysozyme; s, staphylococcal nuclease; ▲, carboxypeptidase A; ○, concanavalin A; X, myoglobin; ▲, PHBG, $5^\circ$ in H$_2$O; O, PHPG, 25°C in H$_2$O; □, PHEG, 25°C in H$_2$O; and P, a partially denatured protein.
all-or-none model for helix to coil transition of a chain molecule in solution. Later theories allowed for the existence of both helix and coil segments within the same molecule (49-55). This dissertation will make use of the theory of Zimm and Bragg (50) in which each residue of a polypeptide chain is allowed to exist in two states, α-helical or random coil. If the $\phi$, $\psi$ angles of a residue fall within the narrow range of the α-helix, the residue is designated $h$, for helical. Residues with any other $\phi$, $\psi$ angles are therefore called $c$, for coil. A further designation arises from the difference between a helical residue that is hydrogen bonded to the third preceding residue and one which is not so bonded. It is assumed that such hydrogen bonds are the only intrachain hydrogen bonds that are formed. The difference in designation of the two types of $h$ residues will become clear by further explanation of the Zimm-Bragg treatment.

By the rule set above the first $h$ residue in a helical segment will be non-hydrogen bonded and it follows that segments of less than three $c$ residues will not be allowed. In statistical mechanics a quantity, $Q(n)$, called the conformational partition function must be calculated. $Q(n)$ contains the statistical weight of every one of the infinite conformations possible for a long chain molecule in which each residue can have an infinite number of states and is calculated according to the following:

$$Q(n) = \prod_{i=1}^{n} \exp(-\varepsilon_i/kt) dq$$

Equation 3
$\epsilon_i$ is the free energy of the $i$th residue in any one of the infinite conformations a residue can assume. If one assumes that each $\epsilon_i$ is independent and can have only discrete values, then Equation 3 becomes

$$Q(n) = \sum_{j=1}^{m} \prod_{i=1}^{n} \exp(-\epsilon_i/kT)$$

Equation 4

where $m$ is the number of discrete states in which the residue can exist. Zimm and Bragg assign $\exp(-\epsilon_i/kT)$, the statistical weights of a residue as follows:

1. The quantity unity for every $c$.
2. The quantity $s$ for an $h$ that follows an $h$.
3. The quantity $\sigma s$ for every $h$ that follows $\mu$ or more $c$'s.
4. The quantity $0$ for every $h$ that follows less than $\mu c$'s.

The quantity $\mu$ for the $\alpha$-helix is three due to the size of the hydrogen bond loop. The significance of (4) above is that residues that are within a hydrogen bond loop cannot exist as coil, so must be helical. Therefore, no segment of less than three coil residues is said to exist. The factor, unity, is arbitrarily assigned to the coil state. The factor, $s$, is the measure of the relative weight of a helical to nonhelical residue. Finally, the $\sigma s$ factor indicates the great decrease in statistical weight of a residue that is helical and unbonded. This residue is the first residue of a helical segment and $\sigma<1$. The factor $s$ not only is the statistical weight of a bonded $h$ residue, it is the equilibrium constant for the propagation of a
helical segment. Calculated values of s for the homopolymers of naturally occurring amino acids range from 0.6 to 1.4 (56-64). The existence of the first residue of a helical segment is of low probability because each first residue has neither the low enthalpy imparted by a hydrogen bond nor the high entropy associated with the coil state. The smaller the value of σ, the fewer number of helical segments that will exist. The values of σ reported in the literature for polyamino acids vary from $10^{-6}$ to $10^{-2}$ (56-64).

If one represents a homopolypeptide as a string of letters either h or c and considers all arrangements of n such letters, then using Zimm-Bragg statistical weights the equation for Q(n) becomes the following:

$$ Q(n) = 1 + \sum_{\ell} \left( \sigma^\ell \prod_{k=0}^{(n-2)/2} \frac{(k-1)! (n-k-2)! s^k}{\ell! (\ell-1)! (n-k-2)!} \right) $$

Equation 5

The term $(n-2)/2$ is the largest integer less than $(n-2)/2$. The use of Q(n) as defined above yields the following expression for $\theta(n)$, the average fraction of hydrogen bonds per molecule of length n.

$$ \theta(n) = \frac{1}{(n-3)} \frac{d \ln Q(n)}{d \ln s} $$

Equation 6

At large values of n and small values of σ ($<10^{-3}$), the value for $\theta(n)$ becomes indistinguishable from the fraction of helical residues present, $f_h$, which will be called fraction helix. Expressions for the
average number of helical segments, \( u(n) \), and the average number of residues per helical segment, \( L(n) \), can also be determined by use of \( Q(n) \).

\[
v(n) = \frac{\delta \ln Q}{\delta \ln \sigma}
\]

Equation 7

\[
L(n) = \frac{(n-3)\theta(n)}{u(n)}
\]

Equation 8

Since \( \mu = 3 \) for the equations above, each helical segment must be preceded by a minimum of 3 coil residues. If \( \mu = 1 \) then the term \( n-3 \) in Equations 6 and 8 would become \( n \). Coil segments of any length could be possible. The number of statistical weights each residue could have would then become three instead of four. The calculation of \( Q(n) \) can now be easily performed by matrix methods. Again, setting \( \mu = 1 \) is reasonable if \( n \) is large and \( \sigma \) is small.

Matrix methods have been devised to deal with the problem of calculating \( Q(n) \). The following scheme provides for all statistical weights that can be encountered in a polypeptide chain when \( \mu = 1 \).

\[
\begin{array}{c|c|c|c|c}
  & l & c & h \\
\hline
  c & 1 & \sigma s \\
  h & 1 & s \\
\end{array}
\]

The terms 1, \( s \), and \( \sigma s \) are statistical weights for residue \( i \) which can be either \( h \) or \( c \) preceded by residue \( i-1 \) which can be either \( h \) or \( c \).

This schematic leads to the following statistical weight matrix:
The conformational partition function can now be calculated for a homopolypeptide of length \( n \) by the following method:

\[
Q(n) = \begin{bmatrix} 1 & 0 \end{bmatrix} U^n \begin{bmatrix} 1 \\ 1 \end{bmatrix} \quad \text{Equation 9}
\]

The term \( U^n \) is the statistical weight matrix multiplied times itself \( n \) times and \( \begin{bmatrix} 1 \\ 0 \end{bmatrix} \) and \( \begin{bmatrix} 1 \\ 1 \end{bmatrix} \) are row and column matrices. The value of \( Q(n) \) calculated by this method will be identical to that calculated by Equation 4.

If there exists a matrix

\[
\begin{bmatrix}
\lambda_1 & 0 \\
0 & \lambda_2
\end{bmatrix}
\]

such that

\[ U = A \begin{bmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{bmatrix} A^{-1} \]

where

\[ A^{-1} A = E = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \]
then the expression for $Q(n)$ becomes the following:

$$Q(n) = [1 \ 0] A^{-1} \begin{bmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{bmatrix} A A^{-1} \begin{bmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{bmatrix} A \ldots A^{-1} \begin{bmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{bmatrix} A^n [1]$$

Equation 10

Equation 10 can be reduced to

$$Q(n) = [1 \ 0] A^{-1} \begin{bmatrix} \lambda_1 \\ 0 \end{bmatrix} A \begin{bmatrix} 1 \\ 1 \end{bmatrix}$$

Equation 11

$A^{-1}$ and $A$ are found to be

$$\begin{bmatrix} \lambda_1 - s & \lambda_2 - s \\ 1 & 1 \end{bmatrix}$$

and

$$\begin{bmatrix} 1 & s - \lambda_2 \\ -1 & \lambda_1 - s \end{bmatrix} \frac{1}{\lambda_1 - \lambda_2}$$

respectively. The values of $\lambda_1$ and $\lambda_2$, called the eigen values of $U$, are determined to be

$$\lambda_1, \lambda_2 = \frac{1 + s \pm \sqrt{(1 - s)^2 + 4gs}}{2}$$

Equation 12

$\lambda_1 > 1$, and $\lambda_2 < 1$.

$$Q(n) = \frac{(1 - \lambda_2)\lambda_1^n + (\lambda_1 - 1)\lambda_2^n}{\lambda_1 - \lambda_2}$$

Equation 13
\[
\theta(n) = \frac{s}{n} \frac{[n(1-\lambda_1)\lambda_2^{n-1} - \lambda_1^n] + [n(1-\lambda_2)\lambda_1^{n-1} + \lambda_2^n] \lambda_1'}{(1-\lambda_2)\lambda_1^n + (\lambda_1-1)\lambda_2^n} - \frac{\lambda_1' - \lambda_2'}{\lambda_1 - \lambda_2}
\]

Equation 14

As \( n \to \infty \), the limiting value of Equation 14 is

\[
\theta(n) = f_h = \frac{\lambda_1 - 1}{\lambda_1 - \lambda_2}
\]

Equation 15

and

\[
u(n) = \frac{\lambda_1}{\lambda_1 - \lambda_2}
\]

Equation 16

and

\[
L(n) = \frac{n(1 - \lambda_1)}{(\lambda_1 - \lambda_2)} \frac{\lambda_1}{1 - \lambda_2}
\]

Equation 17

These limiting equations can be used to interpret helix coil transitions of very long polypeptide chains. (See Figure 7.)

The value of \( f_h \) is experimentally accessible and \( \sigma \) and \( s \) have been determined (56) for homopolypeptides by obtaining \( f_h \) over a range of temperatures and molecular weights. This calculation is performed by first assuming \( \sigma \) is independent of temperature and using curve fitting data. After a fit is obtained \( \sigma \) is allowed to be a function of temperature until a new \( \sigma \) and \( s \) are determined and the iteration process can be performed until a final value of \( \sigma \) and \( s \) are determined.
Figure 7. Values of \( f \) calculated by Equation 14 plotted against increasing degree of polymerization (number of residues per molecule). The limiting values for \( n \to \infty \) are shown as horizontal lines. The values of \( \sigma \) are indicated on the graph.
Unfortunately, most homopolypeptides of the naturally occurring amino acids do not meet the requirements of being soluble and undergoing a helix-coil transition within the temperature range of liquid water. Therefore, a modification of the Zimm-Bragg treatment was developed by Scheraga and co-workers (65) to determine the values of $\sigma$ and $s$ for the naturally occurring amino acid residues. This "guest-host" technique employs a random copolymer of a water soluble, nonionic amino acid residue, the host, with the naturally occurring amino acid residue, the guest. In order to be used the copolymer must meet the requirements of solubility and melting temperature mentioned above. The host residues are those of the homopolypeptides PHPG and PHBG. (See Appendix 1.) These homopolypeptides meet the requirements of solubility and transition temperature. A current list of $\sigma$ and $s$ for amino acid residues is listed in Table 3.

The conformation of homopolypeptides in solution can be changed by changing system conditions of temperature, pressure and solvent composition. Investigations of these transitions have been performed experimentally and theoretically (10,42,44,66-82,89,90). The effects of temperature, the neutral salts, $\text{CaCl}_2$ and $\text{NaClO}_4$, and of charged detergents on the conformation of homopolypeptides in aqueous solutions is of major concern in this dissertation.

Various theories concerning the effect of salts on polypeptide conformation in solution have been put forth (83-88). The two most elementary directions that these theories can take are that the salt has a direct effect on the polypeptide (84,91) or that the
Table 3. σ and s values of amino acids determined to date in water at 20°C (57-63,131-137)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>σ x 10^4</th>
<th>s</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>0.1</td>
<td>0.59</td>
<td>57</td>
</tr>
<tr>
<td>Ser</td>
<td>0.75</td>
<td>0.76</td>
<td>58</td>
</tr>
<tr>
<td>Val</td>
<td>1</td>
<td>0.93</td>
<td>62</td>
</tr>
<tr>
<td>Glu, pH 8.0</td>
<td>6</td>
<td>0.97</td>
<td>63</td>
</tr>
<tr>
<td>PHPG</td>
<td>2.8</td>
<td>0.97</td>
<td>56</td>
</tr>
<tr>
<td>PHBG</td>
<td>6.8</td>
<td>1.02</td>
<td>56</td>
</tr>
<tr>
<td>Ala</td>
<td>8.0</td>
<td>1.07</td>
<td>59</td>
</tr>
<tr>
<td>Phe</td>
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<td>1.08</td>
<td>61</td>
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<tr>
<td>Leu</td>
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<td>1.14</td>
<td>60</td>
</tr>
<tr>
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<td>63</td>
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<td>Arg</td>
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<tr>
<td>Thr</td>
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</tbody>
</table>
salt caused changes in the structure of the solvent (91). Direct salt effects on the polypeptide can be explained by interactions with the peptide backbone or with the side chains. Salts are known to interact electrostatically with charged side chains (92). For example, perchlorate salts cause a coil to helix transition in poly-L-lysine (93). However, the effects on charged side chains are not sufficient to cause the denaturation of native proteins (85,92). Investigations with PHEG, PHPG, and PHBG have shown that the lengthening of the hydrophobic side chain by one or two methylene groups causes significant effects on \( c \) and \( s \) (94). Salts also affect the hydrophobic side chains by increasing their activity, but such action should cause salting out of native state proteins, which is not observed with potassium and sodium salts of \( I^- \), \( NO_3^- \), and \( ClO_4^- \) and the chloride salts of \( Ca^{2+} \), \( Ba^{2+} \), and \( Mg^{2+} \) (84,87,95-99).

The most plausible theory on the disrupting effects by neutral salts on the secondary structures of both native proteins and homopolypeptides is that the salts interact to varying extent with the amide bonds of the peptide backbone (83-88,91). Both cations and anions have demonstrated ability to disrupt secondary structure in polypeptides (84,91). Such cations are believed to bind the carbonyl part of the amide bond (84,91) and anions are believed to interact with the \(-NH--\) part (84,91). Evidence of anion-amide interactions is that large anions bind the uncharged, amide containing polymer, polyvinylpyrrolidone and polyacrylamide; this binding has been attributed to the ion-dipole interaction and van der Waal's forces (95-100).
Salts such as ammonium sulfate have long been used to "salt out" proteins in their native conformation. This effect is very different from denaturation and has been attributed to several properties of salt solutions, among which are water structure changes (84,91) and effects of salts on the internal pressure of the solvent (84,91). The question of whether the two phenomena—salting out and denaturation—are caused by the same interactions, still remains.

Solvent structure changes are an attractive explanation of the effects of salts on protein structure. Salts that destroy tetrahedral water structure such as NaClO₄ denature proteins and salts like (NH₄)₂SO₄ salt-out proteins. However investigations have indicated that some structure forming ions, such as Li⁺, salt-in small polypeptides (84); however, the peptide used in this study, acetyltetraglycine ethyl ester, is virtually incapable of forming a secondary structure unless aggregated. Infrared Raman spectroscopy has shown that the perchlorate ion is a very strong breaker of the hydrogen bond structure of water and this effect is strikingly similar to the effect of a large increase in temperature (101). In the same work it was shown that the perchlorate ion does not form hydrogen bonds to the water molecule.

Circular dichroism has been used to study conformational changes in polypeptides (10,42,78,80). Transitions from α-helix to random coil can be followed by measurement of CD at 222 nm (42). Salts have demonstrated effects on the CD of small polypeptides which are incapable of forming the α-helix due to their small number of
residues (78). Therefore, circular dichroism has been chosen to
study the effects of salts on polypeptide structure.

Detergents have been shown to have effects on both native
protein conformation (102-108) and conformation of homopolypeptides
(72-74). Sodium dodecyl sulfate (SDS) has been used to denature
proteins (102-108) while it has also been shown to induce secondary
structure in positively charged homopolypeptides (72-74). SDS has
little effect on the secondary structure of negatively charged and
neutral homopolypeptides (109-111). The induction of the α-helix in
poly-L-glutamate by dodecylamine has been reported and is included in
this dissertation. The change in charged homopolypeptide conformation
induced by both SDS and docecylamide occurs near a ratio of 1:1 of
detergent to residue and electrostatic binding of the detergent to
the charged side chain has been implicated (112,113).

In this dissertation PHEG, PHPG, PHBG and Ac(Ala)$_3$OMe have
been used to investigate the effects of CaCl$_2$ and NaClO$_4$ on secondary
polypeptide structure. Particular attention has been paid to the
tendency of low concentrations of NaClO$_4$ to cause increase in the
fraction helix of PHEG, PHPG and PHBG. Binding constants for CaCl$_2$
and NaClO$_4$ have been estimated and an explanation of the effect of
NaClO$_4$ on these three homopolymers will be suggested. The effects of
SDS on charged poly-L-histidine and of CTACl and dodecylamine on poly-
L-glutamate have also been investigated.
Chapter II

EXPERIMENTAL

Materials

Sodium perchlorate used for experimental purposes was reagent grade obtained from G. Frederick Smith Chemical Co. Dilutions for spectropolarimetric measurements were made from stock solutions of 2.0 M and 6.0 M. The 6 M stock solution was made by dissolving 24.5 g of NaClO₄ in 20 ml of distilled water and diluting to 25 ml. Calcium chloride was purchased from Matheson, Coleman and Bell, Manufacturing Chemists. A saturated solution at 25°C was prepared. This solution has a concentration of 6.1 M. Further dilutions were made from this stock solution. SDS (abbreviations are listed in Appendix I) was obtained from BioRad Laboratories and recrystallized from water-ethanol by William H. Harrison. A 1% stock solution of SDS (3.47 x 10⁻² M) was prepared from the recrystallized product. Further dilutions were made from this stock solution. Dodecylamine (98%) was purchased from Aldrich Chemical Company, Inc. A 1.0 x 10⁻² M stock solution was prepared by dissolving 0.47 g of dodecylamine in 50 ml of 10⁻² M HCl and diluting to 100 ml. TRIS was obtained from Fisher Scientific Co. All measurements of the effects of dodecylamine on the conformation of poly(Glu) at pH 7.6 were made in 0.1 M TRIS.
Figure 8. Chemical structures of PBG, PHBG, PHPG, and PHEG.
The synthetic peptides used in these studies were all formed from L-amino acids. Poly(Glu) studies as a function of pH were performed without buffer with a pH check immediately before and after each spectrum. The pH measurements were made with a Beckman 3500 pH meter. Cetyl trimethylammonium bromide (CTAB) was obtained from Sigma Chemical Company. The CTAB was converted to the chloride by the following procedure: Approximately 60 ml of 1% CTAB-H2O solution was placed in a segment of 1-inch diameter dialysis tubing. The tubing was tied at both ends with as much air as possible removed as the second end was tied. The dialysis tubing was obtained from Sargent-Welch Scientific Co. It was pretreated by boiling in diluted Na2CO3 followed by storage in propanol. The CTAB was dialyzed against 3 l of 3 M NaCl (reagent grade from Matheson, Coleman and Bell, Manufacturing Chemists) for two days. Excess NaCl was removed by dialyzing against distilled water for one day. The solution remaining in the tube was lyophilized using a dry ice-diethylene glycol monobutyl ether trap. Ultraviolet absorption at 199.5 nm indicated less than 2% bromide in the residue (114). A 0.01 M solution of CTACl was prepared by diluting 0.32 g of CTACl to 100 ml with distilled water. Subsequent dilutions for spectropolarimetric studies were made from this stock solution.

PBG was purchased from Pierce Chemical Company, who reported it to have a molecular weight of 200-400 x 10^3 daltons. Dioxane was purchased from Matheson Scientific Co. and redistilled by Don Clark. The reagent 4-aminobutanol was purchased from Pfaltz Chemical Co.
and was redistilled under vacuum at 71°C using a dry ice-diethylene glycol monobutyl ether trap and a water-cooled condenser. Vacuum was provided by a Scientific Products piston vacuum pump.

PHBG was prepared from PBG by the following modified method of Lothan et al. (110): Three hundred seventeen milligrams of PBG was dissolved in one ml of dioxane in a 50 ml reaction vial. Three milliliters of 4-aminobutanol and an additional 0.5 ml of dioxane was added to the reaction vial. The reaction mixture was stirred magnetically in the sealed vial in a 60°C water bath and allowed to react for one week. The reaction mixture was then poured into 20 ml of chloroform in which the PHBG precipitated as a wax-like substance. The precipitate was washed three times with ether and dissolved in 20 ml of distilled water. The solution was dialyzed against 2000 ml of distilled water which was changed twice daily for five days. It was then filtered through a 1.2 micron Millipore filter and lyophilized using a dry ice-diethylene glycol monobutyl ether trap. The weight of the recovered product was 0.2 g which was a yield of 70%. By ultraviolet absorption measurements at 258 nm the product was shown to contain less than 0.5% benzyl group (115).

PHEG was prepared by J. T. Lo (116). The molecular weight of the PHEG was 200-400 x 10^3 daltons. PHPG was purchased from the United States Biochemical Company. The company claimed the molecular weight to be 7.5 x 10^5 daltons. Poly(His) and Poly(Glu) were purchased from Sigma Chemical Co. The molecular weights were reported by Sigma to be 1.6 x 10^4 and 9.8 x 10^4 daltons respectively. Sigma
indicated the molecular weight of the poly(Glu) was determined by viscosity methods. The oligopeptide, Ac(Ala)$_3$OMe, was obtained from Cyclo Chemical, Division of Travenol Laboratories, Inc.

Unless otherwise stated, the molar concentrations of polypeptide stock solutions were determined by adding the appropriate amount of distilled water to weighed amounts of the polypeptides in a volumetric flask. The concentration of the poly(His) stock solution was determined by amino analysis by Dr. E. W. Blakeney and the poly(Glu) stock concentration was determined by ultraviolet absorption at 197 and 205 nanometers (117). The concentration of amino acid residue used in the CD measurements ranged from $4.4 \times 10^{-5}$ M to $1.8 \times 10^{-3}$ M.

**Optical Activities**

A Durrum-Jasco Model J-20 recording spectropolarimeter was used to measure circular dichroism. Calibration of the instrument was performed by using D-10-camphorsulfonic acid in water. The molar ellipticity of this compound is 7260 deg cm$^2$/dmole at 291 nm at 25°C. Measurements were performed in calibrated quartz cells which had path lengths ranging from 0.1 to 10 mm. Cell temperature was maintained using a circulating water bath connected to a brass water jacket that surrounds the cell during operation. Cell temperature was determined by a YSI Model Y2SC telethermometer with thermocouple placed in the cell before and after each spectrum. Mean residue ellipticity of the polypeptides studied was calculated according to the following formula:
Equation 18

\[ \theta = \frac{fs(E - B)M_{RW}}{1c} \]

\( \theta \) is the mean residue ellipticity in units of deg cm\(^2\)/dmol.
\( f \) is an instrument calibration factor (normally = 1.0).
\( s \) is the scale amplitude factor in millidegrees/cm.
\( E \) is the experimental deflection in centimeters.
\( B \) is the baseline deflection in centimeters.
\( M_{RW} \) is the average molecular weight of the residues of the polypeptide.
\( l \) is the light path in millimeters.
\( c \) is the concentration of the polypeptide in mg/ml.

**Ultraviolet Absorption**

All UV absorption spectra were obtained from a Carey-14 scanning spectrophotometer in 1.0 cm quartz cells at room temperature.

**Calculation of Fraction Helix**

Fraction helix is the accessible experimental quantity in this study. It can be calculated from the following equation:

\[ f_h = \frac{[\theta]_E - [\theta]_C}{[\theta]_H - [\theta]_C} \]

Equation 19

The term \( f_h \) is the fraction helix. \( [\theta]_E \) is the experimental value for mean residue ellipticity at a specified wavelength. \( [\theta]_C \) is the mean residue ellipticity of the completely random coil at the same wavelength, temperature, and solvent conditions. \( [\theta]_H \) is the mean residue ellipticity of a peptide that is completely helical. All calculations of fraction helix in this study make use of the mean residue ellipticity at 222 nm. \( [\theta]_E \), \( [\theta]_H \), and \( [\theta]_C \) were determined at 222 nm. The wavelength 222 nm was selected because the \( \alpha \)-helix
displays a strong negative CD band at that wavelength (42). Peptides believed to be in the random coil conformation show small positive, zero or small negative CD at 222 nm (42,118). The strong negative minimum at 222 nm is believed to be due to the n-π* transition of the amide groups is the α-helical configuration (119).

[θ] has been estimated from the CD spectra of the oligomer Ac(Ala)₃OMe. This peptide is too short to form the first hydrogen bond of the α-helix (78); therefore, it is assumed to have the optical activity of the random coil at all temperatures and salt concentrations. The residue weight is considered to be the molecular weight divided by the total number of amide and ester carbonyl groups present in the oligomer (78). Division of the molecular weight of Ac(Ala)₃OMe by four yields the average weight per chromophore. The values of [θ]₂₂₂ for Ac(Ala)₃OMe at various temperatures compare well with those of PHEG (78,120,121), a polymer that is considered to be almost entirely random coil in aqueous solution (120). Because it is impossible for Ac(Ala)₃OMe to form a helical structure, the salt effects on the random coil polypeptide can be inferred from the effects of salts on this oligomer. The effect of NaClO₄ on Ac(Ala)₃OMe has been investigated (78) and the results from this work have been used to approximate the effects of NaClO₄ on the random-coil polypeptide (Figure 8). Experimental results of the investigation of the effect of CaCl₂ on Ac(Ala)₃OMe at various temperatures were obtained as part of this study. [θ]₀'s for the detergent effect are taken from the plot of the plot of temperature versus [θ]₂₂₂ from the work of Mattice (78).
The mean residue ellipticity of the α-helix was assumed to be $-4.07 \times 10^4 \text{ cm}^2 \text{deg/dmole}$ at 222 nm. This value was taken from the spectrum of poly(Glu) at pH 2.7 at 25°C. This value is slightly greater in magnitude (approximately 10%) than average values of the mean residue ellipticity for completely helical homopolypeptides in water (42,118).

**Calculation of Molal Activities**

When dilution of a stock solution is used to obtain experimental solutions, the new concentrations should be calculated in molal units since determinations of molality of the solutions would require knowledge of the density of both stock solution and solvent. Activity coefficients have previously been calculated for molal concentrations (122); therefore it is necessary to change the experimental molar concentrations to molal concentrations. The relationship between molality and molarity can be expressed as follows:

$$ m = \frac{1000M}{1000d - M \cdot M_w} $$

Equation 20

The terms $M$, $m$, $M_w$ and $d$ are molarity, molality, molecular weight and density respectively. The molarity is known from the experimental dilution of the stock solution. Density was interpolated or extrapolated from tables of densities of electrolyte solutions found in *International Critical Tables* (123). The concentrations from these tables must be converted from percent by weight to molarity for
The term $\nu$ is the number of moles of ions formed by the ionization of one mole of solute. Now, rearranging Equation 25, we have

$$\frac{\delta \ln \gamma_\pm}{\delta T} = \frac{1}{\nu RT^2} \frac{L}{T}$$

Equation 26

which when integrated from $T_1$ to $T_2$ yields the following:

$$\ln \gamma_\pm |_{T=298} = -\frac{1}{\nu R} \int_{298}^{T} \left( \frac{L_2 - T_2 \bar{J}}{T^2} + \bar{J} \frac{1}{T} \right) dT$$

Equation 27

The equation above leads to the following expression for $\ln \gamma_\pm$, which can be used to calculate the new value of $\gamma_\pm$ at any temperature:

$$\ln \gamma_\pm_T = \ln \gamma_\pm_{298} - \frac{1}{\nu R} \left[ \frac{(T-298)(L_2-298\bar{J})}{298T} + \bar{J} \ln \frac{T}{298} \right]$$

Equation 28

The values of $L_{298}$ and $\bar{J}$ up to 2.0 m for CaCl$_2$ have been tabulated (125). However, the values of $L_{298}$ and $\bar{J}$ for NaClO$_4$ have not been calculated and another method had to be found for calculating activity coefficients for NaClO$_4$ at temperatures other than 25°C. The activity coefficients of NaClO$_4$ at freezing from 0.05 m to 1.1 m have been determined (126). By assuming that $L_T$ of Equation 7 is constant, values of $\ln \gamma_\pm$ of NaClO$_4$ can be extrapolated and interpolated from a plot of $\ln \gamma_\pm$ of NaClO$_4$ vs 1/T. Since $\ln \gamma_\pm$ at 25°C and at
use in Equation E2. The conversion from percent concentration to molarity is given by the following equation:

\[
M = \frac{100c}{M_w} \quad \text{Equation 21}
\]

The term \(c\) is the concentration in weight percent defined as

\[
c = \frac{\text{wt. of solute} \times 100\%}{\text{wt. of solute} + \text{wt. of solvent}} \quad \text{Equation 22}
\]

Once molality has been determined the molal activity coefficient for \(\text{CaCl}_2\) and \(\text{NaClO}_4\) at 25°C can be obtained from tables (124,125). The activity coefficients at the experimental concentrations can be interpolated or extrapolated from the information in the table. Values of the activity coefficient, \(\gamma^*_z\), at temperatures other than 25°C can be calculated (125). If \(\overline{L}_T\) is the partial molal heat content at temperature \(T\) and \(\overline{J}\) is the partial molal heat capacity of the solute which is independent of temperature, then \(\overline{L}_T\) and \(\overline{J}\) are related as follows:

\[
\overline{J} = \left(\frac{\delta\overline{L}}{\delta T}\right)_p \quad \text{Equation 23}
\]

which upon integration yields:

\[
\overline{L}_T = \overline{L}_2 + \overline{J}(T - T_2) \quad \text{Equation 24}
\]

By definition:

\[
\overline{L}_T = -uRT^2\left(\frac{\delta \ln \gamma^*_z}{\delta T}\right)_p \quad \text{Equation 25}
\]
freezing of NaClO$_4$ is known, then the values of ln $\gamma_\pm$ of NaClO$_4$ at other temperatures can be estimated. In order to calculate values of ln $\gamma_\pm$ of NaClO$_4$ at concentrations greater than 1.1 m the values of ln $\gamma_\pm$ of NaClO$_4$ at 70°C were extrapolated to high concentrations and in conjunction with ln $\gamma_\pm$ at 25°C used to extrapolate and interpolate values at other temperatures.

**Procedures**

The effects of salts and temperature on the conformation of synthetic homopolypeptides was investigated by obtaining the following CD spectra or measurements at 222 nm:

1. Determination of $[\theta]_{222}$ of 0.30 mg/ml solution of Ac(Ala)$_3$OMe in H$_2$O and in 1.0 M, 2.0 M, 4.0 M, and 5.4 M CaCl$_2$ at 5°C, 25°C, 40°C, and 65°C. The measurements were performed using a 1.0 mm light path and CD scale of 5 m°/cm.

2. Determination of $[\theta]_{222}$ of 0.031 mg/ml PHPG, 0.030 mg/ml PHEG, and 0.030 mg/ml PHBG in H$_2$O and in the following concentrations of NaClO$_4$: 0.05 M, 0.1 M, 0.15 M, 0.2 M, 0.3 M, 0.4 M, 0.8 M, 1.0 M, 1.5 M, 2.0 M, 3.0 M, 4.0 M, 5.0 M, and 5.4 M. The temperatures of the measurements of PHPG were 5°C, 15°C, 25°C, 40°C, 55°C and 70°C. Temperatures of the PHEG measurements were 5.5°C, 25°C, 40.5°C, and 64°C. Temperatures of the PHBG measurements were 5°C, 25°C, 39°C, 55°C, and 71°C. PHEG data were not collected at 5.0 M NaClO$_4$. A 1.0 cm light path and 5 m°/cm scale were used.
3. $[\beta]_{222}$ was determined for 0.310 mg/ml PHPG and PHBG in 
$H_2O$ and 0.12 m, 0.30 m, 0.16 m, 1.25 m, 2.5 m, 4.0 m, 5.4 m, and 6.5 m 
$CaCl_2$. CD data for PHBG were obtained at 5°C, 40°C, and 65°C. The 
measurements of PHPG were made at 5°C, 25°C, 40°C, and 65°C. At 25°C 
CD data for PHBG were determined for 0.1 m, 0.2 m, 0.5 m, 1.1 m, 
1.6 m, 2.2 m, 2.5 m, 3.0 m, 3.3 m, and 3.8 m $CaCl_2$. The CD scale was 
5 m°/cm and the light path was 1.0 mm.

4. CD spectra of poly(His) in the presence of SDS and 
poly(Glu) in the presence of CTACl and DA were under the following 
conditions: a. Poly(His) spectra were run in $H_2O$ (10^{-3} M HCl, 
pH 3.4) and in the same solvent with SDS in molar ratios of 0.3, 0.6, 
0.93, 1.08, 1.86, and 9.3. The poly(His) concentration was 
0.90 x 10^{-4} M. The spectra were run at 25°C with CD scale 2 m°/cm 
and light path 2.0 cm. b. Poly(Glu) spectra were obtained for 
4.4 x 10^{-5} M poly(Glu) in aqueous TRIS (0.01 M) at pH 7.6 and in the 
same solvent with the following concentrations of DA: 2 x 10^{-4} M, 
1 x 10^{-4} M, and 5 x 10^{-6} M. The spectra of poly(Glu) in $H_2O$ as a 
function of pH were obtained for pH values: 3.1, 3.62, 4.52, 6.23, 
6.25, 6.75, 7.15, 7.6, 10.8, 5.84, and 6.27. CD spectra of 
poly(Glu) were obtained in 1.0 x 10^{-2} M and 3.0 x 10^{-4} M CTACl at 
pH values: 3.0, 4.0, 5.0, 6.3, 6.6, 7.8, 9.0, 10.0 and 2.75, 3.2, 
3.9, 4.3, 6.3, 3.5, 5.0 respectively. The temperature, light path, 
and scale for all of the poly(Glu) spectra were 25°C, 1.0 cm, and 
5 m°/cm respectively.
Chapter III

RESULTS

Activity Coefficients of NaClO₄ and CaCl₂

Figure 9 shows the dependence of the activity coefficients on concentration of the salt at 25°C. (See Chapter II for source of activities.) The activity coefficient of NaClO₄ does not become greater than 1.0 with a minimum about 2 m, but that of CaCl₂ has a minimum at about 0.6 m and increases to beyond 40 at high concentrations.

Circular Dichroism of PHBG

The CD spectrum of PHBG in H₂O from 260 nm to 200 nm at temperatures from 5°C to 70°C is shown in Figure 10. At low temperatures this polymer exhibits large negative bands at 208 nm and 222 nm and is rising to a large positive band below 200 nm. There is a local maximum at 214 nm which is located between the two negative bands. The large negative band at 222 nm is due to the n-π* electronic transitions of the peptide units that are in the α-helical conformation (42). The 208 nm negative band and the large positive band below 200 nm arise from π-π* electronic transitions of parallel and perpendicular electronic bands respectively (9). As the temperature is raised, the two negative bands decrease in intensity and the rise at 200 nm disappears, making the local maximum at 214 nm more distinct.
Figure 9. Activity coefficient ($\ln \gamma ^{\pm}$) plotted against molality of $\text{CaCl}_2$ and $\text{NaClO}_4$. 
Figure 10. CD spectra of PHBG in H₂O at 5°C, 25°C, 40°C, and 70°C plotted as [θ], deg-cm/dmol versus wavelength, nm.
CaCl₂ has the same effect as a large increase in temperature on the spectrum of PHBG. The effect of various concentrations of CaCl₂ on the CD spectrum of PHBG is represented in Figure 11. On the other hand, addition of NaClO₄ does not cause effects that parallel the effects of increasing temperature. Representative effects of NaClO₄ on the CD of PHBG at 25°C are demonstrated in Figure 12. The negative bands at 208 nm and 222 nm are increased in NaClO₄ concentrations below 1.5 m, but decrease in higher concentrations of NaClO₄, as illustrated by spectra at 1 m and 4 m NaClO₄.

The effect of increasing concentrations of CaCl₂ is illustrated in Figure 13. There is a decrease in [θ]₂₂₂ from 0 m to 1.25 m CaCl₂ which becomes sharper between 1.25 and 2.5 m CaCl₂. The slope of the curve decreases to zero at higher concentrations and may become negative beyond 4 m CaCl₂. Figure 14 is a plot of [θ]₂₂₂ versus temperature at selected concentrations of CaCl₂. The slope of the curve is positive below 4 m, about zero at 4 m, and negative at 5.6 m CaCl₂. The graph of [θ]₂₂₂ of PHBG in solutions of NaClO₄ is given in Figure 15. There is a rapid decrease in [θ]₂₂₂ from 0 m to 0.5 m NaClO₄. The slope goes to zero between 0.5 m and 2.0 m NaClO₄. From 2.0 m to 7.3 m NaClO₄ the curve appears to be linear with a positive slope. Figure 16 shows the dependence of [θ]₂₂₂ on temperature at selected concentrations of NaClO₄. The slope of these curves is positive at all concentrations of NaClO₄.

If molal concentrations of CaCl₂ are converted to molal activities (see Figure 17), the [θ]₂₂₂ curve loses the sigmodial
Figure 11. CD spectra of PHBG at 25°C in H_2O, 1.2 m CaCl_2, and 5.1 m CaCl_2 plotted as [θ] deg-cm/dmol versus wavelength, nm.
Figure 12. CD spectra of PHBG at 25°C in H₂O, 2 M NaClO₄ and 4 M NaClO₄ plotted as [θ] deg·cm/dmol versus wavelength, nm.
Figure 13. \([\theta]_222^\circ\text{deg-cm/dmol, of PHBG versus molality of CaCl}_2\) at 5°C (●), 25°C (▲), 47°C (●), and 65°C (●).
Figure 14. $[\theta]_{222}^\circ$, deg-cm/dmol, of PHBG versus temperature in H$_2$O (⋆), 1.2 m CaCl$_2$ (●), 2.5 m CaCl$_2$ (●), and 5.6 m CaCl$_2$ (★).
Figure 15. $[\theta]_{222}^\circ$, deg-cm/dmol, of PHBG versus molality of NaClO$_4$

at 5°C (■), 15°C (X), 25°C (○), 39°C (©), and 71°C (●).
Figure 16. $[\theta]_{222}^\circ$, cm-deg/dmol, of PHBG versus temperature, °C, in H$_2$O (•), 0.83 m NaClO$_4$ (♦), 2.2 m NaClO$_4$ (○), and 6.6 m NaClO$_4$ (★) at 25°C.
Figure 17. $[\theta]_{222}^*$, deg·cm/dmol, of PHBG as a function of the activity of CaCl$_2$ at 5°C (*), 25°C (●), 47°C (●), and 65°C (○).
Figure 18. $[\theta]_{222}$, deg-cm/dmol, of PHBG as a function of the activity of NaClO$_4$ at 5°C (•), 25°C (★), 39°C (☆), 55°C (○), and 71°C (▲).
Figure 19. CD spectra of PHPG at 5°C, 25°C, 40°C, and 70°C plotted as \([\theta]\), deg-cm/dmol versus wavelength, nm.
Figure 20. CD spectra of PHPG at 25°C in H₂O, 1.2 m CaCl₂ and 4.0 m CaCl₂ plotted as [θ], deg-cm/dmol, versus wavelength, nm.
Figure 21. $[\theta]_{222}^\circ$ deg-cm/dmol, of PHPG as a function of molality of CaCl$_2$ at 5°C (●), 25°C (□), 40°C (◆), and 65°C (◆).
quality. The curve's slope goes from a positive value at low activities to zero around an activity of 10. (Not shown in Figure 17.) Beyond activity 50 the slope at higher temperatures is negative. The plot (Figure 18) of $[\theta]_{2\overline{2}2}$ versus molal activity of NaClO$_4$ is similar to the molal concentration plot. The initial slope is slightly more negative with other features remaining essentially the same.

**Circular Dichroism of PHPG**

Figure 19 shows that the CD spectrum of PHPG has two negative bands in the region from 260 nm to 200 nm. These bands at 208 nm and 222 nm are not as intense as those of PHBG at the same temperature. As the temperature increases the negative bands show a decrease in intensity. The local maximum between the two negative bands increases with increasing temperature while the 208 nm band becomes less intense and falls off toward a minimum below 200 nm.

CaCl$_2$ affects the spectrum of PHPG by causing effects similar to a large increase in temperature. With increasing concentrations of CaCl$_2$ the spectrum of PHPG undergoes the changes described for increased temperature. At even higher concentrations the negative band at 208 nm disappears completely to be replaced by a positive band at 214 nm. However this maximum itself decreases with increasing temperature and concentrations of CaCl$_2$. (See Figure 20.) The effects of CaCl$_2$ on $[\theta]_{2\overline{2}2}$ is plotted in Figure 21. There is an increase in $[\theta]_{2\overline{2}2}$ up to about 3 m CaCl$_2$ and then a decrease at higher concentrations. There is a slight sigmoidal character to the increase
in [$\theta$]$_{222}$ with increasing molality of CaCl$_2$. If the CD at 222 nm is plotted against activity (Figure 22), the sigmodial character of the curve is changed to monotonic increase in intensity, with increasing activity, until the slope is zero and then a decrease in intensity at activities greater than 10 m. The effect of temperature on [$\theta$]$_{222}$ at selected concentrations of CaCl$_2$ is given in Figure 23. At low concentrations of CaCl$_2$ the slope is positive, but it becomes negative at very high concentrations of CaCl$_2$.

The effect of NaClO$_4$ on the PHPG spectrum is shown in Figure 24. The effect is similar to the effect on PHBG. There is an increase in intensity of the negative bands at 222 nm and 208 nm. This effect reaches a maximum at 1 m to 2 m NaClO$_4$. The effect of concentration of NaClO$_4$ on [$\theta$]$_{222}$ is plotted in Figure 25. There is a relatively sharp decrease in [$\theta$]$_{222}$ up to about 1 m to 2 m NaClO$_4$ followed by a less sharp increase that appears to be linear from 2 m to 7 m NaClO$_4$. Increasing the temperature moves the curve up on the graph and flattens it out slightly. The plot of [$\theta$]$_{222}$ against activity (Figure 26) shows a curve similar to the molality curve. The initial and final slopes are slightly sharper due to decrease in unit size. The effect of temperature on [$\theta$]$_{222}$ at various concentrations of NaClO$_4$ is shown in Figure 27.

**Circular Dichroism of PHEG**

The effect of temperature on the CD spectrum of PHEG is demonstrated in Figure 28. The spectrum at low temperatures has a
Figure 22. $[\theta]_{222}^\text{deg-cm/dmol}$ of PHPG as a function of activity of CaCl$_2$ at 5°C ($\triangle$), 25°C ($\star$), 40°C (O), and 65°C (•).
Figure 23. $\theta_{222}$, deg-cm/dmol, of PHPG versus temperature, °C, for PHPG in H₂O (●), 1.2 m CaCl₂ (■), and 4.0 m CaCl₂ (♦).
Figure 24. CD spectra of PHPG in H₂O, 2 m NaClO₄, and 4 m NaClO₄ at 25°C plotted as [θ]₂₂₂, deg·cm/dmol, versus wavelength, nm.
The graph shows the variation of \( \theta \) with wavelength (nm) for different solutions. The solutions are labeled as \( \text{H}_2\text{O} \), 4M, and 2M. The x-axis represents the wavelength in nm, ranging from 200 to 260, while the y-axis represents \( \theta \) in \( 10^{-4} \) units, ranging from -3 to 1.
Figure 25. $[\theta]_{222}^{2}$, deg-cm/dmol, of PHPG as a function of the molality of NaClO$_4$ at 5°C (■), 15°C (○), 25°C (X), 40°C (△), 55°C (●), and 70°C (□).
Figure 26. $\theta_{222}$, deg-cm/dmol, of PHPG as a function of activity of NaClO$_4$ at 5°C (✩), 15°C (●), 25°C (▲), 40°C (○), 55°C (■), and 70°C (★).
Figure 27. \( \theta_{222} \), deg-cm/dmol, of PHPG as a function of temperature,  
\( ^\circ \text{C} \), in H\(_2\)O (△), 0.83 m NaClO\(_4\) (●), 2.2 m NaClO\(_4\) (●), and  
6.6 m NaClO\(_4\) (●).
Figure 28. CD spectra of PHEG at 5°C, 25°C, 40°C, and 70°C
plotted as [θ], deg-cm/dmol, versus wavelength, nm.
large negative band at 198 nm, a smaller positive band at 214 nm to 216 nm, and a very small negative band at 230 nm to 232 nm. The negative band at 198 nm and the positive band at 214 nm are both decreased in intensity by increasing temperature. The negative band at 230 nm is increased by heating. The positive band at 214 nm becomes a local positive at sufficiently high temperatures. The CD spectrum of PHEG from 260 nm to 200 nm arises from peptide bonds in the random coil conformation (94). Assignment of specific electronic transitions that lead to the features of the random coil spectrum has not yet been achieved. The positive band at 214 nm has been attributed to the n-π* transition of disordered peptides (138,139), but other reports indicate that the rotary strength of the disordered n-π* transition is negative (140). Other investigators attribute the 218 nm positive band to n-π* transitions (141).

The effects of NaClO₄ on PHEG are similar to its effects on PHPG as demonstrated in Figures 29, 30, and 31. Although the aqueous spectrum of PHEG and PHPG at the same temperature are quite different, addition of NaClO₄ causes the CD at 222 nm to decrease and leads to the formation of a negative band at 222 nm. The positive band at 214 nm and the small negative band both disappear. The molal and molar plots show the sharp decrease in [θ]₂₂₂ followed by a less sharp increase in this CD at concentrations greater than 2 m for the 5.5°C spectra. The higher temperature curves reach a minimum at about 2 m but do not rise at higher concentrations. Temperature versus [θ]₂₂₂ graphs for PHEG (Figure 32) show a negative slope in H₂O and very low
Figure 29. CD spectra of PHEG at 25°C in H₂O, 2 m NaClO₄, and 4 m NaClO₄ plotted as [θ], deg-cm/dmol, versus wavelength, nm.
Figure 30. $[\theta]_{222}$, deg-cm/dmol, of PHEG as a function of the molality of NaClO$_4$ at 5.5°C (X), 25°C (o), 40.5°C (e), and 64°C (•).
Figure 31. $[\theta]_{222}^\circ$, deg-cm/dmol, of PHEG as a function of the activity of NaClO$_4$ at 5.5°C (☆), 25°C (★), 40.5°C (★), and 64°C (●).
Figure 32. $\theta_{222}$, deg·cm/dmol, of PHEG as a function of temperature, °C, in $H_2O$ (●), 0.83 m NaClO$_4$ (○), 2.2 m NaClO$_4$ (●), and 7.3 m NaClO$_4$ (‡).
NaClO₄ concentrations, a positive slope in intermediate concentrations of NaClO₄ and zero slope at high concentrations of NaClO₄.

Circular Dichroism of Ac(Al)₃OMe

[θ]₂₂₂ of Ac(Ala)₃OMe is plotted against molality of CaCl₂ in Figure 33. There is a sigmodial decrease in [θ]₂₂₂ over the range in which the activity coefficients of CaCl₂ increase rapidly (1 m to 3 m). When the concentration is expressed as activity, the curve becomes monotonic decreasing (Figure 34). The effects of increasing temperature on the CD of Ac(Ala)₃OMe at selected concentrations of CaCl₂ is shown in Figure 25.

Circular Dichroism of Poly(His)

The CD spectrum of poly(His) at pH 3.4 and temperatures from 15°C to 60°C is shown in Figure 36. The major feature is a large positive band at 220 nm. The spectrum falls off to a minimum somewhere below 200 nm. There is an indistinct small negative band at about 235 nm. This spectrum is quite different from the PHEG spectrum, but has been assigned as the poly(His) random-coil spectrum (8). The difference between the poly(His) random-coil CD spectrum and that of random-coil PHEG may arise from optically active electronic transitions of the imidazole groups of the side chains.

The effect of increasing concentrations of SDS on the spectrum of poly(His) at pH 3.4 is shown in Figure 37. The effect is striking. The large positive band at 220 nm disappears and a large negative band at 224 nm to 226 nm is formed. At high SDS concentrations the lower
Figure 33. [θ]_{222}^{222}, deg·cm/dmol, of Ac(Ala)₃OME as a function of the molarity of CaCl₂ at 5°C, 25°C, 40°C, 47°C, 65°C, and 70°C.
Figure 34. \([\theta]_{222}^\circ\), deg·cm/dmol, of Ac(Ala)_3OMe as a function of activity of CaCl₂ at 5°C, 25°C, 40°C, and 65°C.
Figure 35. $[\theta]_{222}^\text{deg-cm/dmol}$ of Ac(Ala)$_3$OMe as a function of temperature, °C, in H$_2$O (○), 1 M CaCl$_2$ (☆), 2 M CaCl$_2$ (●), 4 M CaCl$_2$ (★), and 5.4 M CaCl$_2$ (●).
Figure 36. CD spectra of poly(His) at 15°C, 25°C, 40°C, and 60°C plotted as \([\theta]\), deg·cm/dmole, versus wavelength, nm.
Figure 37. CD spectra of poly(His) at 25°C in SDS solutions in which SDS/Histidyl = 0.30, 1.08, and 9.3, plotted as [θ], deg-cm/dmol, versus wavelength, nm.
spectrum rises to a maximum below 200 nm. The intermediate SDS concentrations yield spectra that are in various stages of the transitions from the aqueous spectrum to the high SDS concentration spectrum. The high SDS concentration spectrum resembles that of uncharged (non-protonated) poly(His), a structure which has variously been assigned as a left-handed α-helix (142), a right-handed α-helix (70), an ordered conformation different from the right-handed α-helix (143), and the β-structure (8). Figure 38 shows the effect of various ratios of SDS to residue concentration of poly(His) on [θ]220 of that polymer. The major features of the graph is that the steepest slope occurs at a ratio of 1:1.

Circular Dichroism of Poly(Glu)

The CD spectrum of poly(Glu) is dependent on solution pH. Figure 39 shows spectra at three different pH's. The spectrum of the almost completely uncharged poly(Glu) at low pH is typical of the α-helix and is similar to the spectrum of PHBG at low temperature (42). It has two large negative bands at 222 nm and 208 nm and is rising to a large positive below 200 nm. The spectrum of poly(Glu) at pH 10.8 is similar to that of PHEG at the same temperature. It shows the small positive band at 214 nm and negative bands at 230 to 232 nm (very low intensity) and one below 200 nm (very high intensity). At intermediate pH there is a transition between these two spectra. Figure 40 shows this transition by graphing [θ]222 versus pH of poly(Glu) in H₂O, CTACl, and DA. The molar ratios of CTACl:poly(Glu)
Figure 38. Dependence of $[^2]_{220}$ of poly(His) on log (SDS/Histidyl) ratio at 25°C.
Figure 39. CD spectra of poly(Glu) at 25°C in H$_2$O at pH 10.8, pH 6.75, and pH 3.6 ($-10^{-3}$ M HCl) plotted as [θ], deg-cm/dmol, versus wavelength, nm.
Figure 40. pH dependence of $[\theta]_{222}$ of poly(Glu) in $H_2O$ (○), and in $3 \times 10^{-4} M$ (▲) and $10^{-2} M$ (●) CTACl at 25°C.
Figure 41. $[\theta]_{222}$, deg-cm/dmol, of poly(Glu) as a function of
log {[dodecylamine]/[glutamyl]} at 25°C.
are 68:1 and 230:1 and the ratio of DA:poly(Glu) is 230:1. CTACl causes the transition to become very sharp near the pK of the Glu carboxyl group, indicating a destabilization of the slightly charged helix by CTACl. DA causes the transition from helix to coil to occur at a higher pH than in water alone. In the region between the transition in water and the transition in DA solution, the DA stabilizes the helix. A pH of 7.6 was chosen to illustrate the transition from random coil to helix caused by the DA. Figure 41 is a graph of $[\theta]_{222}$ versus the log of the ratio of DA to residue concentration of poly(Glu) at pH 7.6. As in poly(His)/SDS the greatest slope occurs at a ratio of 1:1.
Chapter IV

CALCULATIONS

Fraction Helix

Fraction helix has been calculated according to the description in Chapter II. Figures 42a and 42b show fraction helix of PHBG versus log activity and molality of CaCl$_2$ at several temperatures. In Figure 42b the lowest temperature curve decreases monotonically to near zero at about 4 m. At higher temperatures there is a similar decrease in fraction helix of PHBG with increasing concentrations of CaCl$_2$. At all temperatures the fraction helix increases at concentrations higher than 4 m. Figures 43a and 43b illustrate the effect of CaCl$_2$ on fraction helix of PHPG. The effects are similar to the effects of CaCl$_2$ on PHBG. The fraction helix reaches zero at about 2 m CaCl$_2$ and increases to measurable helix beyond 4 m CaCl$_2$ (Figure 43b). The zero points on this graph do not indicate zero fraction helix, but fraction helix so low that it is beyond the ability of the instrument and method to measure. Any measure of fraction helix less than 0.01 cannot be used for calculations and will be shown as zero on any graphs. Fraction helix versus activity of NaClO$_4$ has been plotted for PHBG, PHPG, and PHEG at several temperatures and are shown in Figures 44, 45, and 46 respectively. For each of these polymers, the fraction helix first increases and then decreases beyond 2 m, a behavior that mimics inversely the decrease and then increase in $[^9]_{222}$. 
Figure 42a. Fraction helix, $f_{h}$, of PHBG as a function of activity of CaCl$_2$ at 5°C (•), 25°C (○), 47°C (☆), and 65°C (★).
Figure 42b. Fraction helix, $f_h$, of PHBG as a function of the molality of CaCl$_2$ at 5°C (●), 25°C (★), 47°C (●), and 65°C (★).
Molality CaCl$_2$ vs. $f_i$. 

Points indicate data points for various molalities of CaCl$_2$, with different symbols representing different conditions or measurements.
Figure 43a. Fraction helix, $f_h$, of PHPG as a function of the activity of CaCl$_2$ at 5°C (●), 25°C (★), 40°C (☆), and 65°C (▲).
Figure 43b. Fraction helix, \( f_h \), of PHPG as a function of molality of \( \text{CaCl}_2 \) at 5°C (●), 25°C (☆), 40°C (▲), and 65°C (○).
Figure 44. Fraction helix, $f_h$, of PHBG as a function of the activity of NaClO$_4$ at 5°C (●), 25°C (☆), 39°C (○), 55°C (★), and 71°C (□).
Figure 45. Fraction helix, $f_{h}$, of PHPG as a function of the activity of NaClO$_4$ at 5°C (●), 15°C (★), 25°C (○), 40°C (▲), 55°C (☆), and 70°C (●).
Figure 46. Fraction helix, $f_h$, of PHEG as a function of the activity of NaClO$_4$ at 5.5°C (★), 25°C (○), 40.5°C (◊), and 64°C (●).
Zimm-Bragg Parameters

The Zimm-Bragg parameters have been discussed in Chapter II. Figure 47 shows how fraction helix is related to $s$ at several values of $\sigma$. Decreasing $\sigma$ causes the transition from helix to coil (coil to helix) to be very sharp with respect to values of $s$. As $\sigma \to 0$ the transition becomes a step function which is discontinuous at $s = 1$. If $\sigma$ is infinitely large the curve is a straight line through fraction helix equal 0.5. Since the weight of a helix initiating residue is infinite when $\sigma$ is infinite, the number of initiator residues would be maximized—a condition that occurs when every other residue is helical.

The values of $s$ and $\sigma$ for PHBG and PHPG have been calculated by Scheraga and coworkers (56). This group used ORD measurements and the Moffit-Yang equation to calculate fraction helix. Table 4 gives these parameters for several temperatures.

Fraction helix is related to $s$ and $\sigma$ in a manner that can be determined for the very high molecular weight polymers by employing the limiting equation for fraction helix (Equation 15) in the following manipulations:

$$f_h = \frac{\lambda_1 - 1}{\lambda_1 - \lambda_2}$$

Equation 15
Figure 47. Fraction helix, $f_h$, as a function of $s$ at (a), $\sigma = 10^{-5}$; (b), $\sigma = 10^{-4}$; (c), $\sigma = 10^{-3}$; (c) $\sigma = 10^{-2}$; (e), $\sigma = 10^{-1}$; and (f), $\sigma = \infty$. 
Substituting for $\lambda_{1,2}$

$$f_h = \frac{(s+1) + [(s-1)^2 + 4\sigma s]^{\frac{1}{2}} - 2}{(s+1) + [(s-1)^2 + 4\sigma s]^{\frac{1}{2}} - (s+1) + [(s-1)^2 + 4\sigma s]^{\frac{1}{2}}}$$

$$f_h = \frac{(s-1) + [(s-1)^2 + 4\sigma s]^{\frac{1}{2}}}{2[(s-1)^2 + 4\sigma s]^{\frac{1}{2}}} \quad \text{Equation 26}$$

$$(2f_h - 1)[(s-1)^2 + 4\sigma s]^{\frac{1}{2}} = s - 1$$

$$(2f_h - 1)^2[(s-1)^2 + 4\sigma s] = (s - 1)^2$$

$$(s - 1)^2 = \frac{\sigma s (2f_h - 1)^2}{(f_h - f_h^2)} \quad \text{Equation 27}$$

Letting

$$a = \frac{\sigma (2f_h - 1)^2}{(f_h - f_h^2)}$$

Then

$$s^2 - (2 + a)s + 1 = 0$$

$$s = \frac{2 + a \pm (a^2 + 4a)^{\frac{1}{2}}}{2} \quad \text{Equation 28}$$

The positive values of the root in Equation 28 lead to $s$ greater than one and the negative value leads to $s$ less than one. The positive and negative values should be used for fraction helix greater than 0.5 and less than 0.5 respectively. If fraction helix equals 0.5, then $s = 1$.

Table 4 gives values of $s$ for PHBC and PHPG calculated from this experimental work and the $\sigma$ parameter of Scheraga and coworkers. These $s$ values are slightly different from the $s$ values of Scheraga. The
different method of calculating fraction helix may be the reason for these differences. By assuming that Scheraga's values are correct, one can calculate σ's different from Scheraga's values to be used for further calculations. These σ's are also listed in Table 4. For subsequent calculations of thermodynamic constants both of the above described methods for calculating s will be used and the results compared.

It has been previously determined that salts have effects on secondary structure in proteins and homopolypeptides (78-80, 144-149). One theoretical treatment of salt effects is a modification of the Zimm-Bragg method. The statistical weight matrix can be expanded to allow for helical and coil residues that bind a specific ligand. In absence of polyelectrolyte effects, the statistical weight matrix can be represented as follows for residue i:

\[
\begin{array}{cccccc}
 i-1 & i & c & c^*L & h & h^*L \\
 c & 1 & K_c[L] & \sigma_s & K_h[L] & \sigma_s \\
c^*L & 1 & K_c[L] & \sigma_s & K_h[L] & \sigma_s \\
h & 1 & K_c[L] & s & K_h[L] \\
h^*L & 1 & K_c[L] & s & K_h[L] \\
\end{array}
\]

Matrix 2

The term \( i - 1 \) indicates that the column signifies the possible residue states of \( i - 1 \) while \( c^*L \) and \( h^*L \) represent ligand bound to coil and helical residues respectively.
Table 4. Values of $s$ and $\sigma$ from von Dreele et al. (56)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Temperature °C</th>
<th>$s^a$</th>
<th>$\sigma \times 10^4^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBG</td>
<td>5</td>
<td>1.040</td>
<td>7.0</td>
</tr>
<tr>
<td>PHBG</td>
<td>15</td>
<td>1.028</td>
<td>6.9</td>
</tr>
<tr>
<td>PHBG</td>
<td>25</td>
<td>1.015</td>
<td>6.8</td>
</tr>
<tr>
<td>PHBG</td>
<td>40</td>
<td>1.000</td>
<td>6.6</td>
</tr>
<tr>
<td>PHBG</td>
<td>55</td>
<td>0.990</td>
<td>6.0</td>
</tr>
<tr>
<td>PHBG</td>
<td>70</td>
<td>0.973</td>
<td>5.6</td>
</tr>
<tr>
<td>PHPG</td>
<td>5</td>
<td>0.990</td>
<td>2.2</td>
</tr>
<tr>
<td>PHPG</td>
<td>15</td>
<td>0.985</td>
<td>2.0</td>
</tr>
<tr>
<td>PHPG</td>
<td>25</td>
<td>0.978</td>
<td>1.8</td>
</tr>
<tr>
<td>PHPG</td>
<td>40</td>
<td>0.975\textsuperscript{b}(0.971)\textsuperscript{c}</td>
<td>1.1\textsuperscript{b}(1.4)\textsuperscript{c}</td>
</tr>
<tr>
<td>PHPG</td>
<td>55</td>
<td>0.980\textsuperscript{b}(0.960)\textsuperscript{c}</td>
<td>0.4\textsuperscript{b}(1.4)\textsuperscript{c}</td>
</tr>
<tr>
<td>PHPG</td>
<td>70</td>
<td>0.985\textsuperscript{b}(0.953)\textsuperscript{c}</td>
<td>0.2\textsuperscript{b}(1.4)\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} All values estimated from a graph of parameter versus $T$.

\textsuperscript{b} Calculated from experiment by von Dreele et al. (56).

\textsuperscript{c} Extrapolated value from early data by von Dreele et al. (56).
The statistical weights assigned to each residue state are determined by the equilibrium scheme in Figure 48 where $K_c$, $K_h$, and $\Sigma K_h$ are binding constants of ligand L to coil, non-initiating helix, and initiating helix residues respectively. The statistical weight of each of the eight possible states of residue $i$ can be determined by multiplying all the equilibrium factors on the arrows between the reference state, $hc$, and the chosen state. The second letter of the two is residue $i$ and that is the residue for which the weight is determined. Since the state (bound or unbound) of residue $i - 1$ does not affect the weight of residue $i$, the statistical weight of all sixteen states in Matrix 2 can be determined. Matrix 2 can be reduced to a four by four matrix.

\[
\begin{array}{ccc}
1 & i & c^* \\
c^* & (1 + K_c[L]) & \Sigma s(1 + \Sigma K_h[L]) \\
h^* & (1 + K_c[L]) & s(1 + K_h[L]) \\
\end{array}
\]

Matrix 3

The symbol * denotes the residue can be bound or unbound.

Each element of Matrix 3 is the sum of the possible weights of bound or unbound, coil or helical residue $i$. By dividing each element by $(1 + K_c L)$, a new and equivalent matrix is generated (Matrix 4).
Figure 48. Equilibrium scheme for binding of a ligand (L) to coil and helix residues.
The upper right element of this matrix can be expressed as

\[
\begin{vmatrix}
1 & h^* \\
(1 + \Sigma K_h[L]) & \frac{s(1 + K_h[L])}{(1 + K_c[L])}
\end{vmatrix}
\]

Expression 1

The following new parameters can be defined:

\[
\sigma' = \frac{\sigma(1 + \Sigma K_h[L])}{(1 + K_h[L])}
\]

Equation 29

and

\[
s' = \frac{s(1 + K_h[L])}{(1 + K_c[L])}
\]

Equation 30

Matrix 4 can now be represented by the following familiar form:

\[
\begin{vmatrix}
1 & h^* \\
1 & \sigma's' \\
1 & s'
\end{vmatrix}
\]

Matrix 5

The new parameters can now be manipulated to predict fraction helix for the high molecular weight approximation.
\[ f_h = \frac{(s'-1) + [(s'-1)^2 + 4\sigma's']^{1/2}}{2[(s'-1)^2 + 4\sigma's']^{1/2}} \]  
Equation 31

This expression in turn can be rearranged to a form similar to 
Equation 27, which can be further rearranged to the following:

\[ f_a = \frac{(s'-1)^2}{\sigma's'} \]  
where  \[ f_a = \frac{(2f_h-1)^2}{(f_h-f_h^2)} \]

Now by resubstituting the expressions for \( \sigma' \) and \( s' \), we have

\[ f_a = \frac{1}{\sigma_s} \left( \frac{1 + K_h[L]}{1 + \Sigma K_h[L]} \right) \left[ s \left( \frac{1 + K_h[L]}{1 + K_c[L]} - 1 \right) \right]^2 \]  
Equation 32

and if \( \Sigma = 1 \), then

\[ f_a = \frac{1}{\sigma_s} \left[ s \left( \frac{1 + K_h[L]}{1 + K_c[L]} - 1 \right) \right]^2 \]  
Equation 33

By adjusting \( K_c, K_h \), and \( \Sigma \) one may fit a number of curves of the 
experimentally calculated \( f_a \) versus the activity of the ligand. A 
computer program using the SAS (Statistical Analysis System) and the IBM 
3033 computer was used for curve fitting (150). The procedure was 
taken from the SAS library and was called NLIN for nonlinear curve 
fitting. This procedure is quite versatile. It has options of 
setting in starting values of the parameters to be determined or it 
will search a field of parameters for minimum starting values for the 
regression part of the program. Any one of three methods of curve
fitting can be chosen. For this work the Gauss-Newton method was chosen for curve fitting. Further SAS procedures incorporated in the method plotted and printed data for quick analysis. See Appendix 2 for typical program and outputs.

The data from the NaClO\textsubscript{4} work could not be fit by Equation 32 or Equation 33. Equation 33 could give a reasonable fit the low concentration portion of the CaCl\textsubscript{2} data. The values of the parameters thus generated were somewhat equivocal and much data had to be deleted to get those values.

Using the values of $\sigma$ from Scheraga and coworkers or generating $\sigma$ from the work of Scheraga and coworkers, one can calculate $s'$ by Equation 28 (assuming $\Sigma = 1$). With $s'$ now a function of fraction helix and constant $\sigma$, the following equation can be used instead of Equation 33 to fit the CaCl\textsubscript{2} data:

$$s' = s \frac{1 + K_h[L]}{1 + K_c[L]}$$  
Equation 34

The increase in helix with increasing low concentration of NaClO\textsubscript{4} can be fit by Equation 34 with $K_h > K_c$. The problem of the decreasing portion of the curve still remains. At this point it is necessary to assume that some factors beyond those already mentioned may be playing a part in the salt effects. A common phenomenon in protein binding of ligands is cooperativity (156) or, as the case may be, anti-cooperativity. To allow for both events, Equation 34 can be modified as follows:
The values of $n_C$ and $n_h$ can be adjusted so that binding to helix or coil can be cooperative ($n > 1$) or anti-cooperative ($n < 1$) (151). By invoking $n_C$ and $n_h$ and using curve fitting techniques, it was found that $n_C$ could remain 1 while $n_h$ turned out to be $0.98 \pm 0.01$ for most of the NaClO$_4$ data. The fit to data as determined by convergence of the program and program generated residual sums was excellent. The CaCl$_2$ data, without the zero points, but using the high activity data was also fit with $n_C = 1$, but with $n_h > 1$ ($n_h = 1.05 \pm 0.04$ for most data). Furthermore the data for CaCl$_2$ could not be fit setting $n_h = 1$ and allowing $n_C$ to be less than one.

The data and generated curves are plotted in Figures 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, and 59. The following equation is assumed to be the proper one to fit all the salt effect data.

$$s' = s \left( \frac{1 + K_h[L]^n}{1 + K_c[L]^{n_C}} \right)$$  \hspace{1cm} \text{Equation 35}

Values of $K_h$, $K_c$, and $n$ were taken from the values generated by the SAS program and are listed in Table 5. The binding constants tend to increase with temperature and the $n$ values show a slight decrease with temperature. $K_h$ and $K_c$ are always strongly correlated and the ratio of $K_h/K_c$ varies from 0.5 to 1.0 for all the combinations of salts and peptides except the one most suspect value (see Table 5).
Table 5. Binding constants at various temperatures

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Temperature °C</th>
<th>$\alpha \times 10^8$</th>
<th>$s$</th>
<th>$K_h$</th>
<th>Standard Error $b$</th>
<th>$K_c$</th>
<th>Standard Error $b$</th>
<th>$n$</th>
<th>Standard Error $b$</th>
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<tr>
<td><strong>Binding of CaCl2</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PHBG</td>
<td>5</td>
<td>7.0$^c$</td>
<td>1.069</td>
<td>0.0593</td>
<td>0.031</td>
<td>0.105</td>
<td>0.0134</td>
<td>0.0108</td>
<td>0.038</td>
</tr>
<tr>
<td>PHBG</td>
<td>25</td>
<td>6.8</td>
<td>1.023</td>
<td>0.0361</td>
<td>0.0134</td>
<td>0.105</td>
<td>0.0134</td>
<td>0.0108</td>
<td>0.038</td>
</tr>
<tr>
<td>PHBG</td>
<td>47</td>
<td>6.0</td>
<td>0.989</td>
<td>0.0938</td>
<td>0.0359</td>
<td>0.133</td>
<td>0.040</td>
<td>0.0156</td>
<td>0.022</td>
</tr>
<tr>
<td>PHBG</td>
<td>65</td>
<td>5.6</td>
<td>0.967</td>
<td>0.0954</td>
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^a Values of σ used in calculations.

^b SAS program generated values.

^c Values of σ taken from von Dreele et al. (56).

^d Values of σ calculated by Equation 27 using values of s from von Dreele et al. (56).

^e Value of s at 47°C agrees with that of von Dreele et al. (56).

^f Temperature independent value of σ assumed in this work.

^g At this temperature constants for PHEG were no imprecise that they were not used for further calculations.
Figure 49. $s'$ values of PHBG plotted against activity of CaCl$_2$ at 5°C (*) and 70°C (*) with the computer calculated curve for $s'$. 
Figure 50. Values of $s'$ for PHPG plotted against activity of CaCl$_2$ at 5°C, 25°C, 40°C, and 65°C with computer generated curves showing the data fit. Horizontal lines represent values of $s'$ that will yield fraction helix values of 1%. Fraction helix can not be measured for $s'$ values at each temperature below the indicated line.
$\sigma = 1.4 \times 10^{-4}$, $40^\circ C$ and $65^\circ C$

$\sigma = 1.8 \times 10^{-4}$, $25^\circ C$

$\sigma = 2.2 \times 10^{-4}$, $5^\circ C$
Figure 51. Fraction helix, $f_h$, of PHBG as a function of activity of NaClO$_4$ at 5°C (●), 25°C (★), 39°C (○), 55°C (★), and 71°C (○). The figure includes the computer estimated curves to show data fit.
Figure 52. Fraction helix, $f_h$, of PHPG as a function of activity of NaClO$_4$ at 5°C (•), 15°C (★), 25°C (○), 40°C (▲), 55°C (★), and 70°C (○). The figure includes computer estimated curves to illustrate the curve fit.
Figure 53. Fraction helix, $f_h$, of PHEG as a function of activity of NaClO$_4$ at 5.5°C (☆), 25°C (○), 40.5°C (●), and 64°C (●). The figure includes the computer generated curves to illustrate the curve fit.
Figure 54. Plot of $\ln K_n$ of CaCl$_2$ binding to PHPG (×) and PHBG (☆) versus $1/T^\circ K$ showing linear regression lines used to calculate $\Delta H_n$. Error bars represent SAS program-generated standard error.
Figure 55. Plot of $\ln K_c$ of CaCl$_2$ binding to PHBG (☆) and PHPG (●) versus $1/T^\circ K$ with linear regression lines used to calculate $\Delta H_c$. Error bars represent SAS program-generated standard error.
Figure 56. Plot of $\ln K_h$ of binding of NaClO$_4$ to PHBG (†), PHPG (*), and PHEG (●) versus $1/T^\circ K$. The linear regression lines used to calculate $\Delta H_h$ are included. The point with the arrow was not included in the calculation of $\Delta H_h$ of PHEG because of probable lack of precision due to extremely low fraction helix at 64°C. Error bars represent SAS program-generated standard error.
Figure 57. Plot of $\ln K_c$ of binding of NaClO$_4$ to PHBG (☆), PHPG (*), and PHEG (●) versus $1/T^\circ K$. The linear regression lines are included in the graph. The point with the arrow (PHEG, 64°C) was not included in the calculations due to lack of precision because of small fraction helix under these conditions. Error bars represent SAS program-generated standard error.
Figure 58. Plot of $n$ values for binding of CaCl$_2$ to PHBG (•), and PHPG (○), and NaClO$_4$ to PHBG (●), PHPG (◆), and PHEG (◇) versus temperature, °C.
Figure 59. Computer estimated values of s for PHXG as a function of temperature: PHBG, CaCl$_2$ (▲), NaClO$_4$ (●); PHPG, CaCl$_2$ (●), NaClO$_4$ (●); PHEG, NaClO$_4$ (★).
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<th>$\Delta H_h$ kcal/mole</th>
<th>$\Delta H_c$ kcal/mole</th>
<th>$\Delta G_h$ kcal/mole</th>
<th>$\Delta G_c$ kcal/mole</th>
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The standard molal enthalpy of binding, $\Delta H_X$ ($x = h$ or $c$), can be determined from the slope of a plot of $\ln K_X$ ($x = h$ or $c$) versus $1/T$ (Figures 55, 56, 57, 58, 59) by the following equation:

$$\left( \frac{d \ln K_X}{dT} \right)_P = -\frac{\Delta H_X}{R} \quad \text{Equation 37}$$

The $\Delta H_X$ values are reported in Table 6. Molal free energy, $\Delta G_X$, can be calculated by

$$\Delta G_X = -RT \ln K_X \quad \text{Equation 38}$$

where $x$ is $h$ or $c$. These values are reported in Table 6. At a particular temperature the molal entropy, $\Delta S_X$, can be calculated

$$\Delta G_X = \Delta H_X - T\Delta S_X \quad \text{Equation 39}$$

and is found in Table 6.

**Detergent Binding**

It is intuitively apparent that a similar treatment to the one above can be used for binding studies of charged detergents to charged polymers. However the statistical weight matrix will have an extra term in it that prevents it from being reduced to the two by two matrix that allows the Zimm-Bragg method to be handled as analytical equations rather than by matrix multiplication. The following slight modification of Matrix 2 can be used for binding of detergents to charged polymers:
In the above matrix the W's are the statistical weighting factors of charged residue \( i \), in which the state of residues \( i-1, i \), is indicated by the subscript. Treatment of data with this matrix will be more difficult and must employ matrix algebra. Such treatment is beyond the scope of this dissertation, but the data collected for poly(His)/SDS and poly(Glu)/CTACl may eventually be analyzed in such a manner.

### Error

A maximum error of 150 m°cm/dmole was assumed for CD measurements. In the bulk of this work, that corresponds to rounding off to the nearest mm of direct measurements of CD data. The SAS program prints out standard error for each parameter estimated. These standard errors are reported in Table 5 and are a good indication of the experimental error in the parameters. The standard error in \( \Delta G \) can be calculated from the standard error of the binding constants and in some cases is very large. Error associated with \( \Delta H \) and therefore \( \Delta S \) is indicated by the error bars in Figures 54 through 57.
For the PHPG work the error in the lines determined is comparable with the standard error. For PHBG and PHEG the error is likely to be greater than standard error.
Chapter V

DISCUSSION

The effects of neutral salts on the conformation of polypeptides have been studied by a number of investigators (78-80, 83, 144-149, 152). The theories that attempt to explain the various effects have been mentioned earlier. This paper will attempt to explain the salt effects of CaCl$_2$ and NaClO$_4$ on poly N-5(hydroxy-N-alkyl) glutamines by showing the preferential binding of the Ca$^{2+}$ or ClO$_4^-$ for either coil or helix residues. Furthermore, the phenomena of cooperativity and anticooperativity in binding to the helix will be asserted. The excellent empirical fit of the data by Equation 36 indicates that the data are not inconsistent with the theory represented by this equation. The calculated values obtained for $K_h$ and $K_c$ will be compared with previously determined values for other similar systems.

The anion ClO$_4^-$ and cation Ca$^{2+}$ bind to amide groups. These ions have been shown to bind to polyacrylamide gel (153). The sodium and chloride ions show close to zero binding to the same gel. The perchlorate anion is believed to bind to amides by charge-dipole interactions (83). This type of interaction is feasible in both helix and coil residues of PHBG, PHPG, and PHEG. These peptides contain an amide group in the side chain also. Therefore, binding per residue
may be more complex than with residues with non-amide-containing R groups. In the studies of NaClO₄ interaction with PHBG, PHPG, and PHEG, binding constants were determined. At all temperatures the binding constants decreased with increasing length of the N-hydroxalkyl group. This tendency compares favorably with the report of Hamabata and von Hippel (154) on the binding of amides and salts. These investigators have shown that the affinity of amides for salts decreases as increasing numbers of methyl substituents are placed around the amide dipole. The following order of decreasing affinity of amides for salts was established: formamide > acetamide = N-methylformamide > N-methylacetamide = N,N, dimethylformamide > N,N, dimethylacetamide. Binding constants for NaClO₄ to polymers PHBG, PHPG, and PHEG range from 0.17 to 10.9 for a helical residue and 0.16 to 9.1 for a coil residue. A reported value of the binding constant of NaClO₄ to the protected tripeptide Ac(Gly)₃OEt at 25°C is 0.75 M⁻¹ (83). Activity coefficients were not employed and the binding constant was determined by solubility studies of Ac(Gly)₃OEt. The binding constant of NaClO₄ to acrylamide gel was determined to be 11.5 x 10⁻² M⁻¹, 7.9 x 10⁻² M⁻¹, and 5.2 x 10⁻² M⁻¹ at 10°C, 25°C, and 40°C, respectively (153). These values were determined by chromatographic methods using polyacrylamide columns.

Investigation by Lotan et al. (44) has shown that aqueous solutions of formic acid cause effects on PHXG which are very similar to those of NaClO₄. Figure 60 represents the effects of formic acid as determined by this group. The curves are similar to
Figure 60. Fraction helix, $f_h$, as a function of mole fraction of water in formic acid. The figure is redrawn from Lotan et al. (44) and represents the fraction helix of PHEG (△, degree of polymerization, 400), PHPG (☆, degree of polymerization, 310), and PHBG (●, degree of polymerization, 630).
those predicted by Equation 36. Lotan et al. (44) indicate that
formic acid can bind to amides by hydrogen binding to the carbonyl
oxygen and amide hydrogen simultaneously. This multiple binding may
be essential for effects of the NaClO₄ type. They, however, ascribe
the effects of formic acid-water mixtures to solvent effects rather
than differential binding to coil and helix.

The binding of CaCl₂ to the amide group is believed by some
investigators to be due to the interaction of the hydrated cation
of calcium with the carbonyl portion of the amide group (83).
Calcium is known to bind strongly to some proteins (155-157). Of
the six oxygen-Ca²⁺ coordinate bonds of each of the two calcium ions
in carp myogen, one of the ligands is an amide carbonyl (155,156).
Van der Helm (157) has shown that with CaCl₂·glycyl glycyl glycine·3H₂O, calcium is seven coordinated with oxygen, three of which
are amide carbonyls. Bond distances of this complex range from
2.296 to 2.503 Å.

In this work calculated values for the binding constant of
CaCl₂ to PHBG and PHPG ranged from 1.6 x 10⁻² m⁻¹ to 7.2 x 10⁻¹ m⁻¹.
Binding constants of CaCl₂ to Ac(Gly)₃OEt and polyacrylamide gel were
reported to be 2.2 x 10⁻¹ M⁻¹ and 5.6 x 10⁻² M⁻¹, respectively (153).
The methods of determining these values are described above. At 25°C,
40°C, and 65°C, the values of Kₜ for PHPG were larger than those for
PHBG. At 5°C, the PHBG values were slightly larger than PHPG values
(PHBG: Kₜ, Kₜ = 0.059 and 0.11, respectively; PHPG: Kₜ, Kₜ =
0.048 and 0.088, respectively). The overall tendency is for
stronger binding of CaCl\textsubscript{2} to the amide with the shorter hydroxyl alkyl group.

The Surface of the \textit{\alpha}-Helix

Binding of protons to uncharged poly(Lys) has been shown to be anticooperative (158) and has been used as a model for binding of a ligand to a rigid linear macromolecule of regular structure (158). This particular model is not applicable to the PHXG binding of salts because the R groups are not charged. The poly(Lys) model of the multiple equilibria approach to cooperativity (160) also requires precise knowledge of bound ligand in order to be useful. For the salt/polymer solutions investigated, this type of direct measurement is impossible.

The possibility of binding of ions to more than one R group amide simultaneously presents an explanation of the relative size of \( K_n \) and \( K_c \) as well as agreeing with surface model theories that have been treated mathematically (159). The surface of a helix presents a uniquely ordered structure to a solution. If a helix surface is "unwrapped" and displayed as a two dimensional plane, the surface can be represented by Figure 61 in which each dot represents an R group. Dotted lines between R groups indicate an interaction with another R group as the groups are bound by a ligand. This interaction can be either cooperative or anticooperative. The model depicted is the "Honeycomb" model. A mathematical treatment of binding to this model is given by Poland (159). Unfortunately this treatment, as
Figure 61. A depiction of the "honeycomb lattice" surface model showing sites that can interact. A star represents an occupied site and connecting bars represent interaction that can lead to cooperativity.
in multiple equilibria treatment, requires precise knowledge of the number of bound ligands in order to be of use in this work. Due to the very high salt concentrations (> 1 m) and very low polymer concentrations (≈ 10^{-4} M) exact binding is not directly measurable. The values of $K_h$ and $K_c$ in Table 5 for all conditions indicate that $K_h$ and $K_c$ are strongly correlated. Other than the one value which is most suspect (see Table 5), $K_h/K_c$ ratios ranged from 0.50 to 0.90 for the CaCl$_2$ binding studies and 1.004 to 1.2 for the investigations of NaClO$_4$ binding. The binding constant of the NaClO$_4$ to the helix is greater than that of the coil. In the helix conformation the back-bond amides are involved in intrachain hydrogen bonding as well as being shielded by other R group atoms. It is obvious that a helix to coil transition of PHXG will expose more amide R groups to the solvent. With the greater number of binding sites thus exposed, it might be expected that $K_c$ will be greater than $K_h$ for an amide binding salt. However, the surface of the helix has properties already mentioned that may enable the ion in question to bind more than one amide group simultaneously. Also, the great decrease in entropy required to position two coil amides for binding one ion would make such binding unfavorable in the coil state. The ClO$_4^-$ is a symmetrical tetrahedron whose bond lengths are 1.56 Å (161) and the distance between oxygen atoms is calculated to be 2.7 Å, and that between outer edges of the oxygen atoms, ≈ 4.1 Å (162). The dimensions of this ion bridge the gap between successive R groups of the backbone or consecutive R groups in the chain. Figure 62
Figure 62. Representation of the size of a perchlorate ion compared with a section of α-helix, illustrating that the perchlorate ion can possibly bind to more than one residue.
illustrates the size of the perchlorate ion relative to a segment of an α-helix. Simultaneous binding of more than one R group amide by the perchlorate anion can lead to the values for $K_h$ which are greater than those obtained for $K_c$.

This sort of reasoning leads to another important point of the helical model. As the number of bound ClO$_4^-$ ions increases, the ability of each new ion to bind two or more sites decreases (159), thus the affinity for each ion decreases as the number of bonds increase. This type of bonding behavior is reflected in a value of $n$ in Equation 36 less than one. In this case $n$ is analogous to the Hill coefficient (151). The very small degree of anticooperativity necessary for the large effects on the helix of PHXG polymers is reflected in the size of $n$ ($\approx 0.98$) for the perchlorate ion binding. Figure 63 represents the helix as a plane with the amide containing side chain represented by arrows whose direction is the direction of the dipole moment of each R group amide. Part A of Figure 63 illustrates a segment of α-helix in which the R groups are randomized. Part B represents the helix at higher concentrations of NaClO$_4$. Finally, the situation in which binding of the next perchlorate ion will be weaker due to the inability of the ion to bind more than one dipole is shown in Part C.

CaCl$_2$ binding to PHXG amide groups may be attributed to coordination type bonding (155-157) or hydrate shell binding (83) as described above. In either case binding to the coil is stronger than binding to the helix (i.e., $K_c > K_h$). The ratio of the binding
Figure 63a. Illustration of the arrangement on a "helix surface" of PHXG of the randomized dipole moments of the R-group with no bound ligand.

Figure 63b. Illustration of the surface of Figure 63a with bound ligand that can bind three dipoles at once. This figure represents low activities of ligand.

Figure 63c. Illustration of the surface of Figure 63a with bound ligand at high activities, a situation in which the next ligand to bind will be bound less strongly.
Figure 64. Representation of the helix surface of Figure 63a with bound Ca$^{2+}$ (●) and Cl$^{-}$ (○). The figure illustrates a way in which saturation of the helix surface at high activities could lead to favorable nearest neighbor dipole interactions.
Figure 65. Plot of $T\Delta S$, kcal/mole, versus $\Delta H$, kcal/mole for salt binding to the PHXG helix, illustrating enthalpy-entropy compensation: PHBG, CaCl$_2$ ($\ast$), NaClO$_4$ (o); PHPG, CaCl$_2$ ($\ast$), NaClO$_4$ (☆); and PHEG, NaClO$_4$ (●).
constants range from 0.5 to 0.9. In the coil conformation there are twice as many amide binding sites, however, calcium binding of two such amides by one ion would be made unfavorable due to the decrease in entropy required when two amides are held at the precise distance for bonding. In the helix multiple binding may occur, producing values of $K_h$ that are close to values for $K_c$. Figure 64 demonstrates a model by which binding of $Ca^{2+}$ to a helix surface could be cooperative. This depicts binding of $CaCl_2$ to helical PHBG at very high activities. At an activity of 100 and binding constants of 0.04 to 0.7, an 80 to 99% saturation of the binding sites is predicted. Figure 64 illustrates a type of binding pattern that could lead to long and short term favorable dipole-dipole interactions of the R group amides that stabilize the helix. The effect would be seen when large portions of the helix surface are saturated by binding ions and would be cooperative.

The models described above are not meant to be precise descriptions of the binding phenomena, but are meant to be one of many plausible explanations of the observed effects of the binding of salts to polypeptide coils and helices.

The Values of $n$

The values of $n$ in Equation 36 are found in Table 6 and are plotted in Figure 59. As the temperature increases, the values are seen to be approaching a value of 1.0 in the range from 5°C to 55°C. Values at 65°C to 70°C have a larger error due to the higher
absorbances of the solutions and less helix fraction at that
temperature. A decrease in cooperativity or anticooperativity with
temperature is feasible since both phenomena depend on an ordered
surface which can become less ordered at a higher temperature. The
greater variability of the n values from the CaCl₂ binding data may
be a reflection of the fewer number of data points used for curve
fitting. The n's determined for binding of NaClO₄ to PHBG were
generally smaller than those for PHPG, which were slightly smaller
than those of PHEG (T < 60°C). The binding of NaClO₄ seems to be
less cooperative as the R group N-hydroxy alkyl moiety becomes longer.

Zero Values of Fraction Helix in CaCl₂ Binding

Figures 42 b and 43 b illustrate the fraction helix of PHBG
and PHPG, respectively, as a function of molality of CaCl₂. At 47°C
and 65°C in Figure 42 b fₕ drops to zero at about 4 m CaCl₂. In
Figure 43 b all the curves from about 2 m to 3 m CaCl₂ reach zero
fraction helix. This phenomenon can be explained by examining
Figures 49 and 50. In these illustrations horizontal lines are
drawn at s' values for each temperature at which fraction helix is
equal to 0.01. The range of the curves below the line correspond to
values of fraction helix which are too low to measure accurately,
therefore producing negative values in some cases. For ranges of
activity where the plot of s' versus activity dips below the
fₕ = 0.01 line, zero or negative values are to be expected. This
method does not predict values of less than 1% helix for PHBG at 5°C.
Estimation of $s$ for PHEG

The value of $s$ for each of the binding situations was estimated by the computer program with the parameters $n$, $K_\text{h}$, and $K_\text{c}$ in order to produce a "best fit." The computer generated values of $s$ were in very good agreement with $s$ values calculated from the fraction helix at zero concentration of the salt for PHPG and PHBG. PHEG has an unmeasurable fraction helix in water solutions and $s$ and $a$ have not been calculated for it. Since NaClO$_4$ induced enough helix formation to determine fraction helix, the information was handled by the SAS program using data at which fraction helix of greater than 0.01 was determined. The program was able to estimate (extrapolate) $s$ from this data using the temperature independent estimation of $1.4 \times 10^{-4}$ for $a$. Values for $s$ were found to be 0.927, 0.853, 0.810, and 0.639 at 5°C, 25°C, 40.5°C, and 65°C, respectively. Such values are not unreasonable for this amino acid.

Thermodynamics of Salt Binding

The standard molal enthalpies and standard molal free energies of salt binding have been calculated from the binding constants. The standard molal entropy has been calculated from $\Delta H_x$ and $\Delta G_x$. Standard molal enthalpy, $\Delta H_x$, increases with decreasing length of the N-hydroxy alkyl group of the homopolypeptides for both salts. The standard molal enthalpy for binding of NaClO$_4$ is negative, near zero, and positive for PHBG, PHPG, and PHEG, respectively. The standard molal enthalpy for binding of CaCl$_2$ has values of $\Delta H_x$ that
are small and positive for PHBG (1-2 kcal/mole) and larger positive values for PHPG (7-10 kcal/mole). The values of $\Delta S_x$ for binding vary directly with $\Delta H_x$. The sign for these values is the same for each polymer as those of the standard molal enthalpy, described above. The values for CaCl$_2$ binding are near zero for PHBG and positive for CaCl$_2$-PHPG binding (21-29 cal deg/mole). The values of $\Delta G_x$ are less variable, ranging from -1.49 kcal/mole to +1.85 kcal/mole for all salt-polmer solutions. The large variations in $\Delta H_x$ and $\Delta S_x$, which compensate each other producing a small change in $\Delta G_x$ over the range of salts and polymers, seem to be an example of the enthalpy-entropy compensation phenomenon described by Lumry and Rajender (163). This phenomenon has been noted to occur in water solutions of many types, especially in water-protein solutions. The temperature at which this phenomenon is noted is characteristically found between 250 and 315°K. A very good example of this compensation is the dissociation of a homologous series of organic acids. For the part of the process which involves solvation, that is, the separation in solution of the charged pair $H^+A^-$, the following relationship has been found:

$$\Delta H_i = \alpha - T_c \Delta S^0_i$$

The term $i$ indicates the species of acid and $\alpha$ is a constant usually taken to be zero. $T_c$ is the compensation temperature. This is the temperature at which this phenomenon occurs. Further examples of this phenomenon are one proton loss from simple alkyl ammonium compounds (164), the thermal unfounding of ribonuclease and trypsin
(163), and the binding of SCN\textsuperscript{−} to methemoglobin (165). In their rather lengthy article Lumbry and Rajender (163) discuss in depth the enthalpy-entropy compensation phenomenon, giving many examples. Figure 65 is a plot of $\Delta H_x$ versus $\Delta S_x$ at 5°C for the binding of NaClO\textsubscript{4} to PHXG. The term $i$ in Equation represents the different lengths of the N-hydroxy alkyl side group and the value of $\alpha$ is near zero.

Since there are only two values of $\Delta S_{x1}^\circ$ and $\Delta H_{x1}^\circ$ for CaCl\textsubscript{2} binding at 5°C, it is impossible to establish compensation, however, when plotted in Figure 65, these two points do define a line nearly parallel to the linear regression of the points for NaClO\textsubscript{4}. The values of $\Delta H_x$, $\Delta S_x$, and $\Delta G_x$ for NaClO\textsubscript{4} binding at 5°C and 25°C are very similar to thermodynamic functions for binding of the thio-cyanate anion to methemoglobin A at pH 7.0 and 20°C (165). It is therefore concluded that the calculated thermodynamic functions of this work are plausible for the systems investigated.

Summary

The data from all the salt binding work done in this study is in excellent agreement with the predictions of Equation 36. This equation predicts values of less than 1% helix at concentrations of CaCl\textsubscript{2} at which zero helix was calculated. It also provides the factor necessary to change the slope of the fraction helix versus activity curves in both NaClO\textsubscript{4} and CaCl\textsubscript{2} binding to PHXG. Equation 36 is an expression of the well-documented phenomenon of cooperativity.
(151,159,160) and can be explained by previously examined lattice models (159). The theory does not require binding of the salt to the coil to be cooperative. By the helix surface model one is able to rationalize the difference of the helix and coil binding constants and their relative sizes. Values of thermodynamic functions vary according to the enthalpy-entropy compensation theory (163) for NaClO$_4$ and these values are similar to the binding of SCN$^-$ to methemoglobin (165). The helix-surface lattice model is well-supported by the work of this dissertation.
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Appendix I

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
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<tbody>
<tr>
<td>Ac(Ala)₃One</td>
<td>N-acetyl-L-alanyl-L-alanyl-L-alanine-O-methyl ester</td>
</tr>
<tr>
<td>CD</td>
<td>circular dichroism</td>
</tr>
<tr>
<td>CTAB</td>
<td>cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>CTACl</td>
<td>cetyltrimethylammonium chloride</td>
</tr>
<tr>
<td>DA</td>
<td>dodecylamine</td>
</tr>
<tr>
<td>deg</td>
<td>degree</td>
</tr>
<tr>
<td>dmol</td>
<td>decimole</td>
</tr>
<tr>
<td>fₜₜ</td>
<td>fraction helix</td>
</tr>
<tr>
<td>Kₜₜ</td>
<td>binding constant of a salt to a coil residue</td>
</tr>
<tr>
<td>Kₘₜₜ</td>
<td>binding constant of a salt to a helical residue</td>
</tr>
<tr>
<td>ΣKₘₜₜ</td>
<td>binding constant of a salt to the first residue in a helical sequence</td>
</tr>
<tr>
<td>m</td>
<td>molality</td>
</tr>
<tr>
<td>M</td>
<td>molarity</td>
</tr>
<tr>
<td>ORD</td>
<td>optical rotatory dispersion</td>
</tr>
<tr>
<td>PBG</td>
<td>poly-γ-benzyl-L-glutamate</td>
</tr>
<tr>
<td>PHBG</td>
<td>poly(N⁵-ω-hydroxybutyl-L-glutamate)</td>
</tr>
<tr>
<td>PHEG</td>
<td>poly(N⁵-ω-hydroxyethyl-L-glutamate)</td>
</tr>
<tr>
<td>PHPG</td>
<td>poly(N⁵-ω-hydroxypropyl-L-glutamate)</td>
</tr>
<tr>
<td>PHXG</td>
<td>PHBG, PHEG, or PHPG</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
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<td>-----------------------------------</td>
</tr>
<tr>
<td>poly(Glu)</td>
<td>poly-L-glutamic acid</td>
</tr>
<tr>
<td>poly(His)</td>
<td>poly-L-histidine</td>
</tr>
<tr>
<td>SAS</td>
<td>Statistical Analysis System</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>TRIS</td>
<td>tris(hydroxymethyl)aminomethane</td>
</tr>
<tr>
<td>[0]</td>
<td>mean residue optical ellipticity</td>
</tr>
</tbody>
</table>
Appendix II. Typical SAS Program Output for Effects of \( \text{HNO}_3 \) on \( \text{PHPG} \) at 40°C*

```
DATA PHPGL;  
INPUT THETA_E THETA_F ACACL2;  
IF (THETA_E=THETA_F) THEN ACACL2=0;  
ACACL2=ACACL2-0.0476;  
ACACL2=ACACL2/2;  
IF P=.01 THEN GOTO P1;  
IF P>.05 THEN GOTO N1;  
GOTO N1;  
N1:  
DATA EVENT;  
DO IN EVENT;  
EVENT;  
DO IN EVENT;  
EVENT;  
EVENTS;  
PROC PRINT;  
RUN;  
```

NOTE: DATA SET WORK.PHPGL HAS 15 OBSERVATIONS AND 7 VARIABLES. 317 OBS/TRY.
NOTE: THE DATA STATEMENTUSED 0.10 SECONDS AND 110 CPU TIME.
NOTE: DATA SET WORK.PHPGL HAS 15 OBSERVATIONS AND 7 VARIABLES. 317 OBS/TRY.
```
TITLE PHRAG.  
MODEL Y=AX+B;  
MODEL Y=B;  
MODEL Y=ACACL2+B;  
TITLE PHRAG.  
```

NOTE: DATA SET WORK.PHPGL HAS 15 OBSERVATIONS AND 7 VARIABLES. 317 OBS/TRY.
```
TITLE PHRAG.  
NOTE: DATA SET WORK.PHPGL HAS 15 OBSERVATIONS AND 7 VARIABLES. 317 OBS/TRY.
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### Statistical Analysis System

```
44 \( n = (1 + 1) \times 2 + \text{size\sigma} + 0.5 \)
45 \( \text{CALC}_1 = (1 + 1) \times 2 + \text{size\sigma} + 0.5 \)
57 \( \text{PRINT} \quad n = 51 \)
```

**Note:**
- Data set WORK. DATA1 has 15 observations and 6 variables, 76% missing.
- The DATA statement used 0.06 seconds and 100K.

```plaintext
44 PROC PLOT
44 PLOT = A
44 OVERLAY
```

**Note:**
- The PROCEDURE PLOT used 0.15 seconds and 136K and printed page 5.

```plaintext
64 PROC PRINT;
```

**Note:**
- The PROCEDURE PRINT used 0.12 seconds and 117K and printed page 6.

**Note:**
- SAS used 117K memory.

**Note:**
- Harri, Goodnight, Dale and Nelwig
- SAS Institute Inc.
- P.O. Box 10066
- Raleigh, N.C. 27605

---

### Nonlinear Least Squares Iterative Phase

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<td>4.00000000</td>
<td>0.00000000</td>
<td>0.00000000</td>
</tr>
</tbody>
</table>

**Note:**
- Convergence criterion met.
PLOT OF PHOSPHATE VS SODIUM PERCHLORATE

LEGEND: A = 1 OBS, B = 2 OBS, ETC.

SYMBOL USED IS *
Appendix III.
SAS Program Plots of CaCl₂ Data and SAS-generated Curve Data

5-axis plots activity of CaCl₂. Y-axis plots fraction helix. The letters A or B represent experimental data and the symbol (*) represents SAS-generated curve.
PHASE 2-SC CALCIUM CHEMISTRY

PLOT OF 

LEGEND: A = 1 PNP, H = 2 PNG, HTR,

PLOT OF CAL Cl-FACCl? SYMBOL USED IS A

0.78

0.68

0.58

0.48

0.38

0.28

0.18

0.08

0.0

A

4

0

12

19

18

11

71

20

79

16

60

89

88

91
PLOT OF PHRCALCI2

LEGEND: A = NAS, R = NAS, ETC.

STANÚ, USED IS A.
PHRG ASC CALCIUM CHLORIDE

PLOT OF FEACL2

LEGEND: A = 1 mEq, B = 2 mEq, ETC.
PLOT OF CALF FEACL2

SYMBOL USED IS *
VITA

Robert William McCord was born in Donaldsonville, Louisiana in 1947 and attended Donaldsonville High School until his graduation in 1965. He attended Nicholls State College in Thibodaux, Louisiana until 1969, at which time he enlisted in the U.S. Navy for four years. After the Navy enlistment, he attended the University of North Florida in Jacksonville, from which he received a B.A. in Natural Science. In 1974 he began graduate school at Louisiana State University in Baton Rouge. After three years of full-time graduate study in Biochemistry, he entered the Tulane University School of Medicine in New Orleans, Louisiana, while continuing as a part-time graduate student at Louisiana State University in Baton Rouge. He was married on August 11, 1979.

He is presently a candidate for the Doctor of Philosophy degree with a Biochemistry major and Chemistry minor.