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Chloride to the rescue

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Chloride to the rescue

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On the fiftieth anniversary of the discovery of the Ser-His-Asp catalytic triad, perhaps the most unusual variation on the textbook classic is described: An incomplete catalytic triad in a hydrolase is rescued by a chloride ion (Fig. 1). Structural and functional data provide compelling evidence that the active site of a phospholipase from *Vibrio vulnificus* employs the anion in place of the commonly observed Asp, reminding us that even well-trodden scientific ground has surprises in store.

Hydrolytic reactions in biology are ubiquitous and essential counterparts of the condensation reactions that drive the assembly of complex molecules from their building blocks. Fifty years ago, two groups studying the structural basis for the hydrolysis of proteins by the archetypical but structurally dissimilar proteases chymotrypsin (1) and subtilisin (2) described what has come to be known as the "catalytic triad." This configuration of Ser, His, and Asp residues, first reported in the context of two very different protein folds, provides the machinery for hydrolysis of peptide bonds, with the His accepting a proton from the Ser hydroxyl group to generate a strong nucleophile and the Asp positioned to stabilize the developing charge on the His. Twenty years later, the same constellation of amino acids was described for a lipase (3), and over the years numerous variations on this biochemistry textbook classic have been observed. For example, Cys can substitute for Ser, or Glu for Asp; in some enzymes, only a dyad is necessary. More recently, the substrate itself has been proposed to provide the nucleophile in a novel variation on the catalytic triad theme: An enzyme that catalyzes the inactivating acetylation of the aminoglycoside antibiotic sisomicin provides the His and Glu of this unconventional catalytic triad, and the substrate -NH_2 is the nucleophile (4).

Now another adaptation is described in this issue of the *Journal of Biological Chemistry*, in which a Cl^- comes to the rescue of an incomplete triad and sits precisely where one would expect the carboxylate of an Asp to be located in the canonical catalytic triad (5). Wan *et al.* had taken interest in the *Vv*PlpA phospholipase from the bacteria *Vibrio vulnificus*, an opportunistic pathogen that unfortunately is occasionally found in oysters harvested from the Gulf Coast of the United States. Some *Vv*PlpA homologs are thought to be virulence factors. However, no structures were available for these proteins, which are annotated as thermolabile hemolysins. Sequence lineups revealed that in *Vv*PlpA and a few other members of this subgroup of the Ser-Glu-Asn-His (SGNH) hydrolase superfamily, which also harbor the catalytic triad first described for chymotrypsin, there is a Gly, rather than the typical Asp. To gain insight into thermolabile hemolysins and to potentially inform antibacterial design, the authors solved the crystal structure of *Vv*PlpA. Their 1.4 Å resolution structure revealed nonprotein spherical density, which could not be attributed to an oxygen atom of a water molecule, as the peak height was consistent with something with more than 8 electrons. After ruling out metal ions (the usual suspects when one is trying to interpret nonprotein, spherical density), the investigators attributed the density to Cl^-. This assignment is consistent with the peak height in the electron density map, an anomalous signal that could be due to Cl^-, and the observed coordination geometry. In addition, the investigators were able to substitute bromide for the chloride and collect the X-ray data at the Br absorption edge. Indeed, the spherical density gave the expected high σ peaks in both the difference Fourier and anomalous maps calculated with the Br-soaked crystal X-ray diffraction data.

The investigators next explored whether the ion plays a role in catalysis. Their data indicate that enzyme activity depends upon chloride concentration. In what can be described as essentially a "reverse" chemical rescue experiment, they also generated the G389D mutant. Consistent with their hypothesis, the activity of the mutant enzyme is not dependent on the presence of chloride. Moreover, the structure revealed the classic catalytic triad with the carboxylate occupying the position of the Cl^- in the WT enzyme.

A quick search of the Protein Data Bank reveals thousands of structures in which Cl^- has been ascribed to a ligand density. Given that chloride concentrations can range from 5 to 200 mM in the cell and that NaCl is often present in buffers, the identification of chloride ions as ligands in protein structures is logical. Although the anion has been suggested to play accessory roles in catalysis in other enzymes (6), this report appears to be the first account of Cl^- serving as an essential part of catalytic machinery. This observation gives one pause to wonder if there might be more instances where Cl^- is an active participant in catalysis, instances that are easily overlooked because the ion is often present in enzyme assays as a buffer component. A more systematic approach to analyzing the depositions in the Protein Data Bank might reveal additional Cl ions suspiciously occupying active site cavities.

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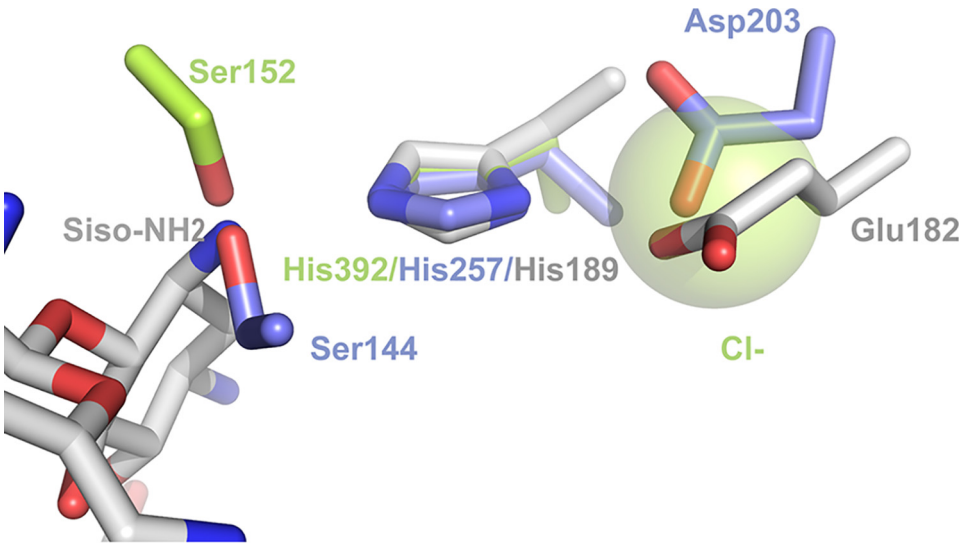

Figure 1. Variations on the catalytic triad theme. The incomplete catalytic triad of *V. vulnificus* PlpA (Protein Data Bank entry 6JKZ) (*lime*, carbon; *red*, oxygen; *blue*, nitrogen) is rescued by Cl^- (*green sphere*). The classic triad as found in triacylglcerol lipase (1TGL) is shown in a *stick rendering* (*pastel blue*, carbon; *red*, oxygen; *blue*, nitrogen). In the acetyltransferase that inactivates sisomicin (6BC3; *gray*, carbon; *red*, oxygen; *blue*, nitrogen), the NH of the antibiotic is positioned in place of the Ser.

As the *Vibrio* phospholipase promotes lysis of the host cells and is a virulence factor, it is tempting to speculate that the need for chemical rescue by chloride might be exploited to engineer a highly specific inhibitor for this particular serine hydrolase by incorporating the halogen into a substrate mimic. Halogens are commonly employed in medicinal chemistry and are staples in drug development (7). For additional specificity, one might also exploit the fact that in this hydrolase structure, which lacks substrate or a substrate mimic, the requisite oxyanion hole, a key feature of catalytic triad active sites, is obscured by an atypical conformation of one the invariant amino acids that define it (Asn-247). Thus, an inhibitor might also incorporate motifs that lock the enzyme into this inactive conformation.

On this fiftieth anniversary of the discovery of the catalytic triad, a wonderfully adaptable and powerful hydrolytic apparatus, we find yet another example of how it can be modified, one in which enzyme activity is rescued by a common chemical component of the cell.

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