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Kinetic characterization of His-tagged CP47 Photosystem II in *Synechocystis* sp. PCC6803

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Abstract

Recently, construction of strains of *Synechocystis* sp. PCC6803 having a His_6 extension (His-tag) of the carboxyl terminus of the CP47 protein has been reported (T.M. Bricker et al, Biochim. Biophys. Acta 1409 (1998) 50; M.J. Reifler et al., in: Garab, Pusztai (Eds.) Proc. XIth International Congress on Photosynthesis, 1998). While these initial reports suggest a minimal impact of the His-tag upon Photosystem (PS) II function, a more thorough analysis of the kinetic properties of the modified complex is essential. This communication reports on a more detailed kinetic analysis to assess possible perturbations of PS II due to the genetic addition of the His-tag on the CP47 protein. It was found that: (1) Patterns of flash O_2 yield exhibited normal period four oscillations and the associated fits of the Kok-Joliot S-state cycling parameters were virtually identical to the wild type; (2) O_2 release kinetics during the S_3–S_0 transition were experimentally indistinguishable from the wild type; (3) S-state decay measurements indicate slightly faster decays of the S_2 and S_3 states compared to the wild type; (4) fluorescence measurements indicate that the kinetics of the forward reaction of electron transfer from Q_A^- to Q_B and back-reactions of Q_A^- with PS II electron donors are similar in the His-tag and wild-type strains. It is therefore concluded that the addition of the His-tag results in a minimal perturbation of PS II function.

Keywords: Photosynthesis; Oxygen evolution; Affinity purification; Histidine tagging

1. Introduction

The histidine tagging (His-tag) technique is a common approach to facilitate the purification of proteins for which the corresponding gene has been cloned. In this technique, the gene for the target protein is engineered to include a sequence of additional histidine residues, typically six, at either the amino or carboxyl terminus of the target protein. The histidine-tagged protein is expressed in a suitable host, usually *Escherichia coli*, and the tagged protein is purified by chromatography on a nickel- or cobalt-chelate matrix, based upon the affinity of the His-tag to ligate the immobilized metal ions.

At issue for any study utilizing this technique, is the extent to which, if any, that the added histidine residues affects the function of the tagged protein relative to the native protein. Recently, the construction and purification His-tagged Photosystem (PS) II particles has been described [1–4]. In these reports, the His-tag was engineered into one of the polypeptides in the native host and the modification thereby

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Table 1
S-State decay cycling parameters[a]

Strain	S-State distribution[b], $S_0/S_1/S_2/S_3$ (%)	Misses[b] (%), α	Hits[b] (%), β	Double hits[b] (%), γ	O_2 release kinetics[c], k^{-1} (s)
Wild type	29/66/5/0	8	89	3	2.0 ± 0.3 ms
HT-3	27/68/5/0	8	90	2	2.1 ± 0.3 ms

[a]Samples were given a series of 20 pre-flashes prior to the 10-min dark period preceding the series of measuring flashes as shown in Fig. 1. The parameters are derived from the averaged data ($n = 3$) presented in Fig. 1.
[b]Numerical analysis of the amplitudes was performed using either 4- or 5-state models as described previously [7,14,15]. Fitting was performed to both the averaged data for the wild-type and HT-3 membrane samples. Fitting to the individual traces before averaging gave essentially the sample results owing to the consistency of the data. The 4- and 5- state models produced essentially equivalent results and no evidence for the presence of an S_{-1} 'super-reduced' state in any of the samples could be demonstrated using the 5-step models.
[c]Estimated from the rise kinetics of the averaged ($n = 20$) O_2 signals shown in Fig. 2b.

permits isolation of the multi-protein complex containing a functional complement of the multiple organic and inorganic cofactors that must be assembled into the complex in order for it to be capable of enzymatic water oxidation. Placement of a His_6 tag at the carboxy terminal end of the CP47 protein in the cyanobacterium *Synechocystis* sp. PCC6803 is reported to allow rapid purification of highly active PS II complexes [3,17]. This is a potentially valuable construction since *Synechocystis* sp. PCC6803 is readily transformable and there are already a large set of existing plasmids for producing defined mutations in the PS II complex of this organism. However, a detailed characterization of the kinetic properties of the electron donor and acceptor sides of the modified enzyme have not been reported. In this communication, a characterization of His-tagged PS II containing a His_6 tag at the carboxy terminal end of the CP47 protein is performed. The analysis shows that the His-tag extension produces little or no apparent alteration in the several kinetic characteristics of the donor and acceptor sides of the enzyme.

2. Materials and methods

2.1. Strains and growth conditions

The strains of *Synechocystis* sp. PCC6803 and HT-3, which carried a His_6 extension of the carboxy terminus of the *psb*B gene, [4] were grown on a rotary shaker in BG-11 media supplemented with 5 mM glucose at 32°C.

2.2. Isolation of O_2-evolving membranes

Isolation of O_2-evolving membranes was performed with modifications of previously described procedures [5]. Cells from 100-ml late logarithmic phase cultures were harvested by centrifugation at 6000 rpm for 10 min at 4°C in a Sorvall GSA rotor. Following harvest, cells were resuspended in approximately 1.2 ml of HMCS buffer (50 mM Hepes, 5 mM $CaCl_2$, 10 $MgCl_2$, 1 M sucrose, pH 7.2) and supplemented with 1 mM each of PSMF, ε-caproic acid and benzamidine to inhibit protease activity, then were transferred to a 2-ml screw-top tube and incubated on ice for 1 h. Cells were broken by shaking with a ratio of 0.6:1 (v/v) of 0.1-mm glass beads using a Mini-Bead Beater machine (Bio-Spec Products). After breakage, the glass beads, unbroken cells and debris were pelleted from the cell homogenate by centrifugation at 3000 rpm for 3 min at 4°C, the membranes were obtained by centrifugation at 70 000 rpm for 30 min at 4°C in a Beckman TLA 100.3 rotor and resuspended in 200 μl HMCS buffer. The membranes were used immediately or were aliquoted and stored at −80°C.

2.3. Flash O_2 yields and decays of S_2 and S_3 states

Flash O_2 yields and decays of S_2 and S_3 states were performed using a bare platinum electrode that permits the centrifugal deposition of samples upon the electrode surface, the procedure was previously detailed [6]. Kinetics analysis of the data was performed according to the exponential method described by Jursinic and Dennenberg [7].

2.4. Fluorescence measurements

Cells in late logarithmic phase of growth were harvested by centrifugation. After being washed with HN buffer (10 mM Hepes (pH 7.0), 30 mM NaCl), the cells were resuspended in HN buffer at a final concentration of 50 μg of Chl ml^{-1}, then the cells were kept under dim light on the shaker at 150 rpm before being used for experiments. Measurement of chlorophyll *a* fluorescence was performed essentially as in reference [8]. A PAM 101 chlorophyll fluorometer with a PAM 103 triggering attachment (Walz Inc., Germany) was used for these experiments. A 3-ml cell suspension at 3 μg of Chl ml^{-1} was incubated in darkness for 5 min before measuring pulses were switched on at 1.6 kHz and the flash given using the automatic switching to 100 kHz measuring pulse prior to applying the actinic flashes.

2.5. Chlorophyll determination

The chlorophyll *a* concentration in this paper was measured in methanol extracts according to Lichtenthaler [9] using the extinction coefficient at 665.2 nm of 79.24 $mg/ml^{-1}\,cm^{-1}$.

3. Results and discussion

The HT-3 strain examined here was constructed, as described previously, by standard recombinant techniques using the strain *Synechocystis* sp. PCC6803 [4]. Essentially, the cloned *psb*B gene, encoding the PS II proximal antennae chlorophyll protein CP47, was modified in vitro by the insertion of six additional His codons (His_6) at the 3′ end of the gene. The resultant plasmid construct was then used to transform a recipient strain containing a deletion in the *psb*B gene such that the transformation replaced the deleted region with His_6-extended version of the *psb*B gene. Thus, the His-tag consists of a His_6 extension of the carboxy terminal end of the otherwise wild-type CP47 protein. According to current models, this places the tag in HT-3 at the cytoplasmic (stromal) side of the photosynthetic membrane [10,11]. For the kinetic characterization of HT-3 and the parental wild-type strain, most of the tests described below were performed using isolated membranes since membranes allow the most accurate measurements of the kinetics of the catalytic cycle using a bare platinum oxygen electrode [6].

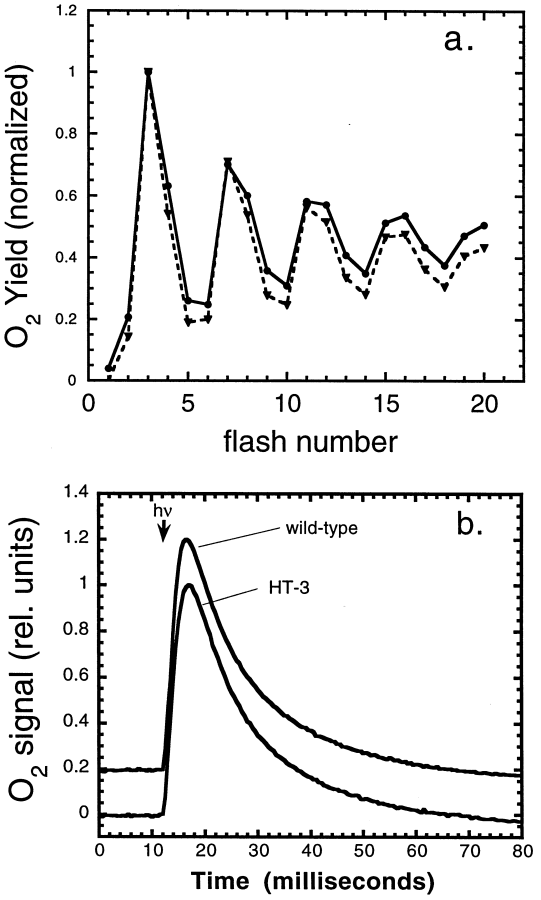

Fig. 1. Flash O_2 yields of isolated membranes. Oxygen production by dark-adapted wild type and HT-3 that have been centrifugally deposited upon the surface of a bare-platinum electrode. (a) The oscillatory pattern of O_2 production as a function of flash number by wild type (●) and HT-3 (▼). Traces represent the average of three measurements. These measurements gave very consistent results with the standard deviations for individual data points less than 5% of the values of the data points. (b) The oxygen signals of wild-type and HT-3 membranes. Signals are the averages of 20 individual flash O_2 signals. The exponential rise times of the depicted signals are given in Table 1. Kinetic analysis of the data was performed according to the exponential method described by Jursinic and Dennenberg [7]. Membranes were centrifuged to the surface of a bare-platinum electrode, dark adapted for 10 min, and given a train of saturating xenon flashes at 4 Hz frequency.

The patterns of flash O_2 yields, shown in Fig. 1, provide information on the cycling of the intermediate oxidation states of the water oxidation complex driven by a sequence of single turn-over light flashes

Table 2
S-State decays[a]

Strain	S_2 decay		S_3 decay	
	Normalized extents (%)	k^{-1} (s)	Normalized extents (%)	k^{-1} (s)
Wild type	21 ± 3	11 ± 2	26 ± 3	13 ± 4
	79 ± 6	246 ± 24	74 ± 5	363 ± 39
HT-3	16 ± 2	6 ± 2	23 ± 4	14 ± 3
	86 ± 13	194 ± 31	77 ± 12	266 ± 25

[a]Results of data shown in Fig. 2. Numerical analysis was performed by fitting to two exponentials described by kinetic constants k_1. Fits were made assuming two exponentially decaying components of the S-state decay, since simpler models gave non-random residuals. The tabulated values are the averages and their associated standard deviations where $n = 3$ and the correlation coefficients (R) of the fits were greater than 0.95. Derived kinetic constants (k) with the characteristic decay times in seconds expressed as k^{-1}.

(for reviews, see [12,13]). During its catalytic cycle, the H_2O-splitting enzyme passes through a series of intermediate oxidation states called S states (S_i, where $i = 0$–4). Oxygen is released from the enzyme during the transition from the S_3 to the S_0 (the S_4 state being an unstable intermediate). Dark-adapted samples relax to the lower S states, primarily to the S_1 state. Consequently, oxygen release from dark-adapted samples does not occur until the third flash and thereafter oscillates with a period of four with the eventual damping of the oscillation as a result of misses and double hits. As shown in Fig. 1a, the patterns of oxygen yield by the HT-3 and wild-type samples are very similar. As shown in Table 1, this impression is validated by numerical analysis of the corresponding yield patterns to obtain the S-state cycling (Kok-Joliot) parameters which reflect the probabilities for advancing the catalytic cycle by a given flash of light as well as the initial distribution of S states in the dark adapted samples [7,14,15]. Therefore, the His-tag does not appear to affect the Kok-Joliot S-state cycling parameters of O_2-evolving enzyme.

Additional information on the functional properties of the H_2O-splitting enzyme can be obtained by evaluating the kinetics of O_2 release during the S_3–S_0 transition [6,7,16]. Fig. 1b and Table 1 compare the kinetics of appearance of O_2 at the surface of the bare platinum electrode released from membranes of the wild type and HT-3 that have been centrifugally deposited upon the platinum electrode surface. Under these conditions the diffusion time of O_2 released from the enzyme is minimized and it is possible to resolve alterations in the rate of the O_2-yielding $S_3 \rightarrow S_0$ transition characteristic of some

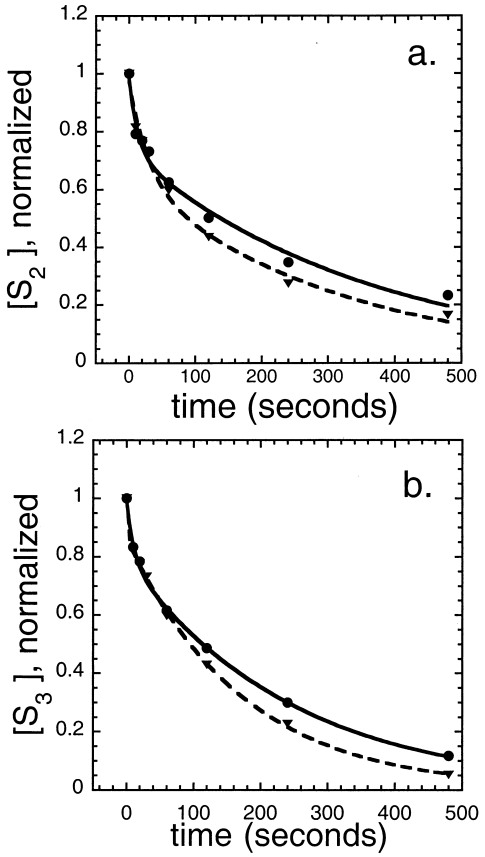

Fig. 2. Decays of the S_2 and S_3 states. Decay of the S_2 state (a) and decay of the S_3 state (b) in membranes of the wild type (●) and HT-3 (▼). Measurements of the lifetimes of the S_2 state were performed by recording the amplitude of O_2 yield on the third flash under conditions varying the time interval between the first and second flashes. Measurement of the lifetime of the S_3 state in each of the different strains was performed by recording the amplitude of O_2 yield on the third flash under conditions varying the time interval between the second and third flashes. Results represent averages as in Fig. 1, and the results were quite reproducible and correspondingly the standard deviations are less than 6% of the values of the data points.

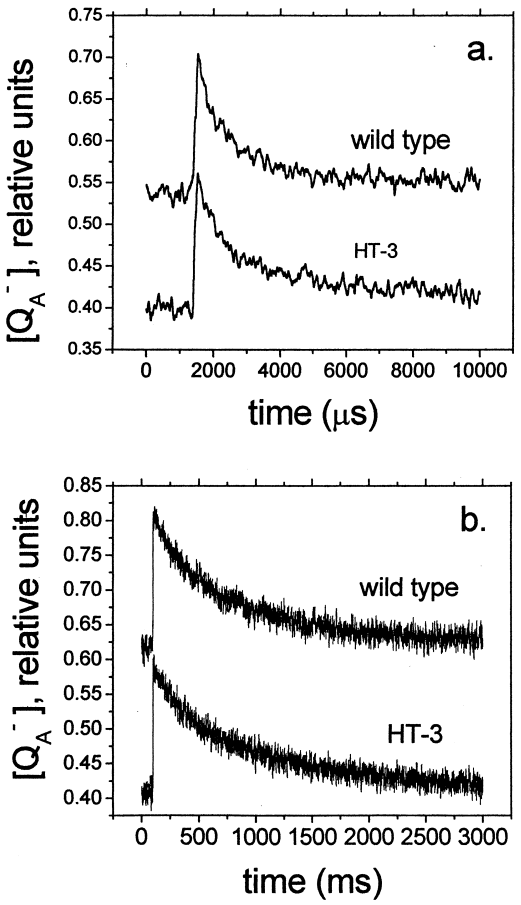

Fig. 3. Fluorescence yield kinetics of Q_A^- reoxidation. The decays of Q_A^- in wild-type and HT-3 cells in the absence of DCMU (a) and the presence of DCMU (b), following a saturating single turnover xenon flash as measured by changes in the yield of chlorophyll *a* fluorescence using a PAM fluorometer with a PAM 103 triggering attachment (Walz Inc.). The cells were incubated in darkness for 5 min before measuring pulses were switched on at 1.6 kHz. Actinic flashes were given using the automatic switching of the triggering attachment which starts the 100 kHz measuring pulses 4 ms prior to the flash. Each trace represents the signal average of 50 recordings performed on the same sample. Different samples produced highly similar results evidenced by the statistical similarity of the numerical fits (Table 3).

mutations affecting the donor side of the PS II complex [6,17,18]. These results indicate that there is little or no change in the rate of oxygen release during the $S_3 \rightarrow S_0$ transition for the His-tagged mutant.

Using double flash techniques it is possible to assess the dark stability of the S_2 and S_3 states in dark-adapted samples that had been flashed to advance them to these more oxidized states of the H_2O-splitting enzyme [6,19,20]. The dark decay of the S_2 and S_3 states corresponds to the re-reduction of the H_2O-splitting enzyme by electrons from, for example, the electron acceptor side of the PS II complex or from the redox active tyrosine, termed Y_D, on the donor side of the complex. Alterations in the decay of the S states have been associated with the absence or loose binding of the extrinsic PS II proteins [20] and with mutations affecting the Mn-binding site [19]. Changes may also be the result of alterations of the electron acceptors of PS II. As shown in Fig. 2a and b, the decays of the S_2 and S_3 states, respectively, are slightly faster in His-tagged PS II membranes compared to the wild type, indicating that the His-tag perturbs the stability of the S_2 and S_3 states (see also Table 2). These differences are greater than the level of experimental error and therefore they represent a small but significant perturbation.

To obtain information regarding possible perturbations of the acceptor side of the PS II complex, kinetic measurements of the decay of fluorescence yield following a single turnover flash were performed in the absence of DCMU (Fig. 3a). These measurements monitor the reoxidation of Q_A^- formed by the flash illumination. In the absence of DCMU, the reoxidation of Q_A^- occurs mainly via forward electron transfer to the Q_B site. Inspection of the plots indicates little difference between the wild-type and HT-3 samples. Correspondingly, kinetic analysis of the data using exponential decay models (Table 3) revealed little difference in the kinetics of fluorescence decay indicating that the reoxidation of Q_A^- in the forward direction is not discernibly affected by the His-tag. The decay of fluorescence yield was also measured in samples in the presence of DCMU (Fig. 3b and Table 3). Under these conditions the decay of fluorescence yield monitors the reoxidation of Q_A^- due to its back-reaction with oxidized forms of PS II electron donors (e.g., the S_2-state of the H_2O-splitting enzyme) and the absence of significant alterations in the kinetics suggests minimal perturbation of corresponding donor–acceptor pairs [8,19,21]. The fact that the Q_A^- back-reaction is not perceptibly altered, whereas the S_2 decay shows a slight, but perceptible acceleration, may be the result of a small alteration of the Q_B site by the His-tag. If this is true, then the putative alteration in the Q_B site is not so great so as to be discerned by

Table 3
Fluorescence decays[a]

	Without DCMU		With DCMU	
Strain	Normalized extents (%)	k^{-1} (μs)	Normalized extents (%)	k^{-1} (ms)
Wild type	49 ± 3	304 ± 36	34 ± 3	310 ± 25
	43 ± 2	440 ± 55	66 ± 3	1236 ± 83
	7 ± 4	1689 ± 171	–	–
HT-3	47 ± 8	377 ± 38	37 ± 9	251 ± 37
	40 ± 5	511 ± 27	63 ± 10	1220 ± 70
	12 ± 3	1536 ± 90	–	–

[a]Data obtained as shown in Fig. 3. Numerical analysis was performed by fitting to three exponentials and two exponentials for the measurements of fluorescence decay in the absence and presence of DCMU, respectively. Numerical fits to extract the kinetic parameters were obtained with correlation coefficients greater than 0.95 for the no-DCMU experiment and greater than 0.99 for the plus-DCMU experiment. The numerical fits were made to at least three separate trials of each experiment and the tabulated values are the

the no-DCMU fluorescence assays monitoring forward electron transfer which primarily represents the average of electron transfer from Q_A^- to produce the fully oxidized (quinone) and singly reduced (semi-quinone) forms of Q_B.

Taken together, the present results suggest that while the His-tag on the carboxyl terminus of the CP47 protein is relatively minimal, clearly future kinetic studies employing its use should take the differences into account. Efforts are presently being directed towards construction of strains that allows transfer of existing PS II mutations into this His-tag PS II genetic background.

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References

[1] M. Sugiura, Y. Inoue, J. Minagawa, FEBS Lett. 426 (1998) 140–144.
[2] M. Sugiura, Y. Inoue, Plant Cell Physiol. 40 (1999) 1219–1231.
[3] M.J. Reifler, D.A. Chisolm, J. Wang, B.A. Diner, G.W. Brudvig, in: G. Garab, J. Pusztai (Eds.), Proceedings of the XIth International Congress on Photosynthesis, Kluwer, Dordrecht, 1998.
[4] T.M. Bricker, J. Morvant, N. Masri, H.M. Sutton, L.K. Frankel, Biochim. Biophys. Acta 1409 (1998) 50–57.
[5] R.L. Burnap, M. Qian, J.R. Shen, Y. Inoue, L.A. Sherman, Biochemistry 3307 (1994) 13712–13718.
[6] M. Qian, L. Dao, R.J. Debus, R.L. Burnap, Biochemistry 38 (1999) 6070–6081.
[7] P.A. Jursinic, R.J. Dennenberg, Biochim. Biophys. Acta 1020 (1990) 195–206.
[8] H.-A. Chu, A.P. Nguyen, R.A. Debus, Biochemistry 33 (1994) 6150–6157.
[9] H.K. Lichtenthaler, Methods Enzymol. 148 (1987) 350–382.
[10] T.M. Bricker, Photosynth. Res. 24 (1990) 1–13.
[11] R.J. Debus, Biochim. Biophys. Acta 1102 (1992) 269–352.
[12] B.A. Diner, G.T. Babcock, in: D. Ort, C.F. Yocum (Eds.), Oxygenic Photosynthesis: The Light Reactions, vol. 4, Kluwer Academic, Dordrecht, 1996, pp. 213–247.
[13] R.D. Britt, in: D. Ort, C.F. Yocum (Eds.), Oxygenic Photosynthesis: The Light Reactions, Kluwer Academic, Dordrecht, 1996, pp. 137–164.
[14] J. Lavorel, J. Theor. Biol. 57 (1976) 171–185.
[15] P.C. Meunier, R.L. Burnap, L.A. Sherman, Photosynth. Res. 47 (1995) 61–76.
[16] P.C. Meunier, R. Popovic, Photosyth. Res. 28 (1991) 33–39.
[17] R. Burnap, J.R. Shen, P.A. Jursinic, Y. Inoue, L.A. Sherman, Biochemistry 31 (1992) 7404–7410.
[18] M. Hundelt, A.M. Hays, R.J. Debus, W. Junge, Biochemistry 37 (1998) 14450–14456.
[19] P.J. Nixon, B.A. Diner, Biochemistry 31 (1992) 942–948.
[20] C. Putnam-Evans, R. Burnap, J. Wu, J. Whitmarsh, T.M. Bricker, Biochemistry 35 (1996) 4046–4053.
[21] R.J. Boerner, A.P. Nguyen, B.A. Barry, R.J. Debus, Biochemistry 31 (1992) 6660–6672.