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Redox Potential of the Primary Plastoquinone Electron Acceptor Q_A in Photosystem II from *Thermosynechococcus elongatus* Determined by Spectroelectrochemistry[†]

Tadao Shibamoto,[‡] Yuki Kato,[‡] Miwa Sugiura,[§] and Tadashi Watanabe*[,‡]

[‡]*Institute of Industrial Science, The University of Tokyo, 4-6-1, Komaba, Meguro-ku, Tokyo 153-8505, Japan, and* [§]*Cell-Free Science and Technology Research Center, Ehime University, Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan*

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ABSTRACT: The redox potential of the primary plastoquinone electron acceptor Q_A, $E_m(Q_A/Q_A^-)$, in an oxygen-evolving photosystem (PS) II complex from a thermophilic cyanobacterium *Thermosynechococcus elongatus* was determined to be -140 ± 2 mV vs. SHE by thin-layer cell spectroelectrochemistry for the first time. The $E_m(Q_A/Q_A^-)$ value obtained here together with the recently determined redox potential of pheophytin (Phe) *a* [Kato et al. (2009) *Proc. Natl. Acad. Sci. U.S.A. 106*, 17365−17370] yields −330 to −370 mV for the free energy change by electron transfer from Phe a^- to Q_A and provides a renewed picture for the energetics on the electron acceptor side in PS II.

Since the "Z-scheme" (*1*) had been proposed, identification of cofactors on the electron-transfer chain and determination of their redox potentials have improved our understanding of the energetics of the primary process in oxygenic photosynthesis (*2*). Not all the redox potentials of the cofactors, however, have been determined, and unapproachable potentials of some cofactors, for example, the primary electron donor P680 of photosystem (PS)[1] II and the electron acceptors of PS I, have been estimated from free energy differences obtained by kinetic analyses on the basis of the measured potentials (*2*). Further, even the experimental potential values exhibit heavy scatters, and each value has often been reported with a non-negligible experimental error (for example, see ref *3*): this has hampered us from depicting the energetics in detail, and such a situation has resulted at least partly from the limited accuracy of chemical titration employed traditionally.

Spectroelectrochemistry can overcome the drawbacks inherent to chemical titration (*4*). We have actually succeeded in measuring precisely the redox potentials of cofactors such as the primary electron donor P700 of PS I (*5*, *6*) and essential cofactors for PS II (cytochrome b_{559} (*7*) and pheophytin (Phe) *a* (*8*)) by spectroelectrochemistry, shedding light on their redox properties buried in previous measurements. In this work, to gain a better insight into the energetics within PS II, we have established a spectroelectrochemical method to determine the redox potential of the primary plastoquinone electron acceptor Q_A, $E_m(Q_A/Q_A^-)$, in PS II.

Photon energy absorbed by peripheral chlorophylls of PS II induces charge separation between P680 and Phe *a*, forming a radical pair $P680^+$ Phe a^-, and subsequently drives forward electron transfer from Phe a^- to the plastoquinone pool bound to PS II via Q_A and the secondary plastoquinone Q_B on the acceptor side, and also hole transfer induced by $P680^+$ simultaneously on the donor side accumulates oxidizing equivalents at the Mn_4Ca-cluster, which catalyzes water oxidation (*2*). On the acceptor side, reduction of Q_A slows the forward electron transfer, and hence promotes fluorescence emission as a dissipation pathway of absorbed photon energy (*2*). In view of this, the fluorescence yield of PS II complexes is neatly influenced by the redox state of Q_A, and thus has often been used as an indicator for the redox titration of Q_A (*3*).

In spectroelectrochemistry, we monitored the fluorescence intensity at 681 nm during a potential journey in a reductive direction for the oxygen-evolving PS II complexes prepared from *Thermosynechococcus elongatus* 43-H (*9*) together with the redox mediators, anthraquinone-2-sulfonate ($E_m = -195$ mV), 2-hydroxy-1,4-naphthoquinone ($E_m = -100$ mV) and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine ($E_m = +300$ mV) in a MES-buffer (pH 6.5) in an optically transparent thin-layer electrode (OTTLE) cell (*5*) (Figure 1A): the fluorescence emitted in line with a very weak monochromatic light (wavelength, 430 nm; light intensity, 0.01 μE s^{-1} m^{-2}) was detected at the backside of the OTTLE cell (see Supporting Information). The fluorescence intensity increased and reached a steady-state in 1000−1200 s by each potential step. The amplitudes of the fluorescence intensity change, assumed as the fraction of the maximum fluorescence change reached by electrochemical reduction being equal to the fraction of Q_A reduced to Q_A^- (Figure 1A), against the electrode potentials gave Nernstian plots as shown in Figure 1B, which obey well a theoretical one-electron process at E_m of −140 mV vs SHE (see also Figure S2, Supporting Information). Such a clear Nernstian behavior of the fluorescence change indicates that the redox states of the mediators used here do not interfere with the measurement, and that a linearity holds between the fluorescence yield and the redox state of Q_A.

After complete reduction of Q_A at a potential of −350 mV, the fluorescence intensity change during the reverse potential journey showed an irreversible process: a portion of the fluorescence intensity induced by the reduced Q_A decreased gradually and

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*To whom correspondence should be addressed. E-mail: watanabe@iis.u-tokyo.ac.jp. Phone: +81 3 5452 6330. Fax: +81 3 5452 6331.

[1]Abbreviations: PS II, photosystem II; Q_A, primary quinone acceptor; HP and LP form, high- and low-potential form; Phe, pheophytin; Chl, chlorophyll; OTTLE, optically transparent thin-layer electrode; MES, 2-[*N*-morpholino]ethanesulfonic acid.

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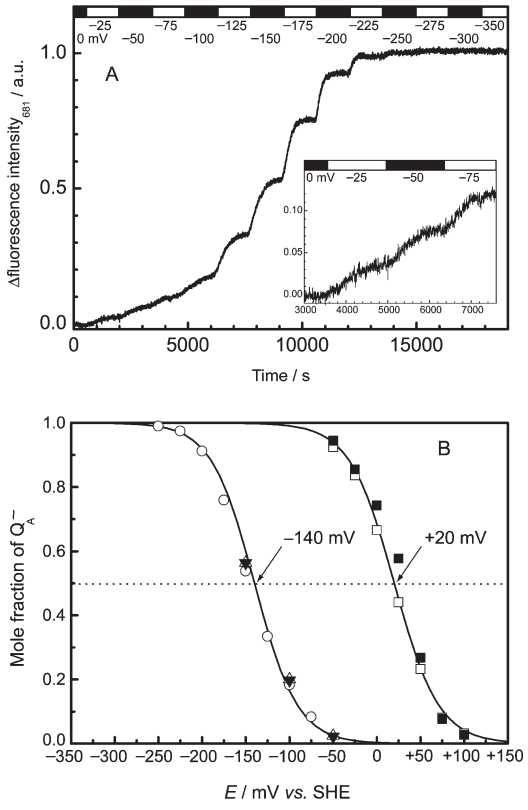

FIGURE 1: Spectroelectrochemical outcome from redox reaction of Q_A in the PS II core complexes from *T. elongatus*: (A) Fluorescence intensity change at 681 nm during a potential journey from 0 to −350 mV for the oxygen-evolving active PS II complexes. The −25 to −75 mV portion is enlarged in the inset; (B) Nernstian plots based on the relative values of fluorescence intensity at 681 nm for intact PS II complexes (circles and triangles) and Mn-depleted inactive PS II complexes (squares): ○ reductive journey from 0 to −350 mV based in Figure 1A; △ reductive journey, ▼ oxidative journey in the range of −150 to 0 mV based on Figure S3B, Supporting Information; □ reductive journey, ■ oxidative journey in the range of +150 to −125 mV based on Figure S4, Supporting Information.

dropped at −125 to −150 mV and the other portion dropped at +25 to +50 mV (Figure S3A, Supporting Information), suggesting that E_m of some portion of Q_A shifted irreversibly to around +25 mV. Meanwhile, reversion of the potential at a more positive potential, not leading to full reduction of Q_A, yielded a reversible fluorescence change as presented by triangles in Figure 1B (see also Figure S3B, Supporting Information). Such irreversible fluorescence change as shown in Figure S3A, Supporting Information, was also observed by Krieger et al. (*3*), who attributed it to the irreversible reduction of the Mn_4Ca-cluster under extremely reducing conditions, resulting in a change in $E_m(Q_A/Q_A^-)$ from the low-potential to the high-potential form. In addition, they demonstrated that inhibition of oxygen evolution from PS II membranes of spinach induces a formation change of Q_A, causing an E_m shift by ca. +150 to +190 mV (*3*, *10*). In view of this, we performed spectroelectrochemical measurements also for the PS II complexes inactivated by NH_2OH washing (Figure S4, Supporting Information; squares in Figure 1B), and this treatment indeed caused a shift of $E_m(Q_A/Q_A^-)$ by ca. +160 mV. This result also ensures that the value of −140 mV is E_m of the "low-potential active form" of Q_A in the PS II complexes from *T. elongatus*.

Four independent spectroelectrochemical runs on the oxygen-evolving PS II complexes led us to conclude that $E_m(Q_A/Q_A^-)$ is −140 ± 2 mV. The high reproducibility and barely scattered plots, obeying well the Nernstian curves (Figure 1B), are a salient feature of spectroelectrochemistry as compared to traditional chemical titrations of $E_m(Q_A/Q_A^-)$. Though the values of $E_m(Q_A/Q_A^-)$ had been reported in a form of four clusters (ca. −300, −100, 0, and +100 mV) (reviewed in ref *3*), by a careful survey of the literature and a systematic evaluation of critical parameters for the scattering, Krieger et al. concluded that E_m of the low potential active form Q_A is −84 ± 16 mV, while E_m for the higher potential inactive form is +65 ± 25 mV (*3*), and these have been considered to be reasonable and are often cited in discussing the PS II energetics (*11*). They claimed that one of the critical parameters influencing $E_m(Q_A/Q_A^-)$ is the effect of redox mediators (*3*), and it was observed that the presence of redox mediators under reducing conditions and/or frozen incubations is prone to causing a change from the low- to high-potential form of Q_A. Though scanning of the electrode potential down to a very low potential as seen in Figure 1B (circles) might indeed have caused a formation change, the observed Nernstian behavior and the reversible fluorescent change at potentials above −150 mV (triangles) supports that −140 mV is the E_m value of the active Q_A. The success in measuring E_m of the active Q_A in this work may have resulted from the use of a nonfrozen samples and an adequate proportion of the redox mediators, as demonstrated by Krieger et al. (*3*), that kept the potential of sample solutions (≈ open-circuit potential) positive of +200 mV.

A value of −84 ± 16 mV for $E_m(Q_A/Q_A^-)$ was reported on PS II membranes from spinach (*3*). Using the same method, Fufezan et al. determined $E_m(Q_A/Q_A^-)$ in the PS II complexes from *T. elongatus* to be +84 ± 24 mV (*12*), which is by ca. +160 mV more positive than that of the active form Q_A in spinach PS II: they argued that it could reflect the difference in species or Mn depletion during titration. In comparison with the present result, the value of +84 ± 24 mV may be the E_m of the inactive form. In any case, our experimental values, −140 mV and +20 mV for the active and inactive forms of Q_A, respectively, are fairly more negative than those of previous studies. One of the causes for such discrepancy may have resulted from the use of mediators, as exemplified by the fact that Krieger et al. obtained a value of −84 mV in the absence of mediators (*3*) while they once obtained a value of −120 mV (error range unknown) by almost the same technique as in the later work except for the use of mediator, where they used methylviologen as mediator (*13*). The presence of redox mediators, indispensable for spectroelectrochemistry of such redox species as the cofactors embedded in protein complexes, may tend to shift the redox potential of Q_A to a certain degree in the negative direction. In view of this, the value of −120 mV for Q_A of spinach might be the counterpart to our value (−140 mV) on *T. elongatus*. Whether the 20 mV difference reflects a species dependence is to be examined in detail through spectroelectrochemical measurements on the Q_A from spinach in the near future.

To discuss the energetics within PS II, it is desirable to use the E_m values of key cofactors obtained under the same experimental conditions. Recently we succeeded in determining the E_m value of Phe *a*/Phe a^- to be −505 ± 6 mV by spectroelectrochemistry under almost similar conditions (*8*); this yields, together with the present result, the energetic relationships within the PS II complexes as depicted in Figure 2. On the forward electron transfer from Phe a^- to Q_A, the free energy difference between [$P680^+$ Phe a^- Q_A] and [$P680^+$ Phe *a* Q_A^-] (ΔG_{PhQ}) is calculated

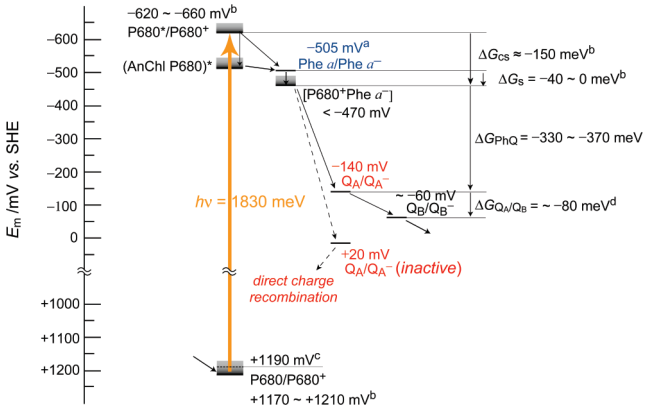

FIGURE 2: Energetics within PS II based on the redox potentials, $E_m(Q_A/Q_A^-)$ and E_m(Phe a/Phe a^-), together with the free energy differences estimated in the literature. The value[a] of E_m(Phe a/Phe a^-) is from ref *8*; for the values[b] of E_m(P680*/P680$^+$), E_m(P680/P680$^+$), ΔG_{CS} and ΔG_S, and the gray boxes indicating uncertainties, see Discussion in ref *8*; the value[c] of E_m(P680/P680$^+$) is calculated from kinetic analytical data (*15*) on the basis of the $E_m(Q_A/Q_A^-)$ value determined in this work; the value[d] of $\Delta G_{QA/QB}$ is from the literature (*16*, *17*). The solid arrows denote the forward electron transfer in oxygen-evolving PS II complexes, while the broken arrows denote the backward (charge recombination) electron transfer for photoprotection of inactivated PS II (see text).

to be −330 to −370 meV on the basis of our results (for the energy level of [P680$^+$ Phe a^- Q_A], see Discussion in ref *8*). This energy difference seems to agree with the values determined by kinetic analyses for the charge recombination of Q_A^- and P680$^+$, −310 meV (*14*) or −340 meV (*15*), more satisfactorily than by using the result of spinach Q_A. The kinetic analyses allowed to estimate E_m(P680/P680$^+$) at ca. +1250 mV by citing $E_m(Q_A/Q_A^-)$ in spinach as a reference potential (*14*, *15*), while the E_m(P680/P680$^+$) value is located from kinetic analytical data together with the $E_m(Q_A/Q_A^-)$ value estimated by us at +1190 mV, which is within the range, +1210 to +1170 mV, calculated from E_m(Phe a/ Phe a^-) and the estimated energy difference between [P680* Phe a] and [P680$^+$ Phe a^-] (ΔG_{CS}) (*8*).

On the electron transfer from Q_A^- to Q_B, the free energy difference was estimated to be −70 meV from fluorescence decay analyses on pea chloroplasts (*16*) and to be −83 meV from thermoluminescence measurements on cyanobacterial cells (*17*). The $E_m(Q_B/Q_B^-)$ value is thus predicted to be ca. −60 mV from the $E_m(Q_A/Q_A^-)$ value determined here, and the electron transfer to Q_B from the inactive Q_A with $E_m(Q_A/Q_A^-) = +20$ mV would be practically prohibited. The inactivation increases the ΔG_{PhQ} value simultaneously by shifting $E_m(Q_A/Q_A^-)$ alone while by keeping E_m(Phe a/ Phe a^-) (*8*) and promotes direct charge recombination between Q_A^- and P680$^+$ as a photoprotection mechanism without reforming the P680$^+$Phe a^- radical pair that possibly generates harmful triplet state (*10*). A computational study suggested that the hydrogen bond between D2-Thr217 and Q_A may be related to this control mechanism for $E_m(Q_A/Q_A^-)$ (*18*).

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SUPPORTING INFORMATION AVAILABLE

Supplementary figures, detailed experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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