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Redox potential of pheophytin *a* in photosystem II of two cyanobacteria having the different special pair chlorophylls

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Table – 1

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Abbreviations used: DCIP, 2,6-dichlorophenol indophenol; DPC, diphenyl carbazide; PS, photosystem; Phe, pheophytin; Chl, chlorophyll.

Author contributions

M.M. designed the research, S.I.A., T.T., H.K., and R.N. performed research, Y.S. analyzed data, and S.I.A., T.T., V.V.K., and M.M. wrote the paper.

Abstract

Water oxidation by photosystem (PS) II in oxygenic photosynthetic organisms is a major source of energy on the earth, leading to production of a stable reductant. Mechanisms generating a high oxidation potential for water oxidation have been a major focus of photosynthesis research. This potential has not been estimated directly but has been measured by the redox potential of the primary electron acceptor, pheophytin (Phe) *a*. However, the reported values for Phe *a* are still controversial. Here, we measured the redox potential of Phe *a* under physiological conditions (pH 7.0, 25°C) in two cyanobacteria having different special pair chlorophylls (Chls): *Synechocystis* sp. PCC 6803, whose special pair for PS II consists of Chl *a*, and *Acaryochloris marina* MBIC 11017, whose special pair for PS II consists of Chl *d*. We obtained redox potentials of −536 ± 8 and −478 ± 24 mV on PS II complexes in the presence of 1.0 M betaine, for *Synechocystis* sp. PCC 6803 and *A. marina*, respectively. The difference in the redox potential of Phe *a* between the two species closely corresponded with the difference in the light energy absorbed by Chl *a* vs. Chl *d*. We estimated the potentials of the special pair of PS II to be 1.20 V and 1.18 V, for *Synechocystis* sp. PCC 6803 (P680) and *A. marina* (P713), respectively, clearly indicating conservation in the properties of water oxidation systems in oxygenic photosynthetic organisms irrespectively of the special pair chlorophylls.

Key words: Photosynthesis, Photosystem II, Pheophytin, Redox titration, Betaine.

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Introduction

Photosynthesis mediates the conversion of solar light energy to chemical bond energy through multistep reactions. Two photosystems (PSs) are present in oxygenic photosynthetic organisms, and these two PSs function cooperatively to capture light energy and drive electron flow. PS II supplies an energy source, i.e. an electron, by water oxidation, and PS I supplies a highly reduced compound, NADPH, to reduce CO_2 to carbohydrates.

Reaction processes in the electron transfer system in photosynthesis are governed by two major factors: the relative geometry and the redox potentials of the electron transfer components; the molecular environment supplied by the amino acid matrix of the components will give a supplemental effect(s). Crystal structures of PS II complexes at atomic resolution have been reported from several laboratories (1-4). Thus, with the exception of some inconsistencies in the water oxidation reaction system, an essential part of the primary charge separation machinery has been characterized (5). The electron transfer mechanisms, in contrast, have not yet been clarified in most cases.

Pheophytin (Phe) *a* is the primary electron acceptor in PS II (6-8), although the primary electron donor of PS II is still controversial (P680 or accessory Chl *a*) (9, 10). These two are not in disagreement with respect to the nature of the primary charge separation but different in the value of rate constants and the question of "transfer to the trap limited" or "trap limited reaction" (5). In this report, we used the term, the special pair, instead of the primary electron donor to avoid confusion on the identification of the primary electron donor in PS II. The redox potential of Phe *a*, E_m(Phe *a*/Phe a^-), is critically important when we consider the water-oxidation system, because it is directly related to the redox potential of the special pair of PS II (8, 11). Since the special pair possesses a very high potential for oxidation of water, direct estimation of this potential by chemical titration is difficult. Instead,

a combination of the measured potential of Phe *a* and theoretical calculations have been used to estimate the potential of the special pair in PS II. The redox potential of Phe *a* was measured for the first time in 1979 in PS II particles from pea and spinach by Klimov et al. and was reported to be −610 ± 30 mV (8). Rutherford et al. reported a very similar value (−604 mV) by EPR spectroscopy using PS II particles from pea (12). Although these values were obtained under non-physiological conditions (at pH 8.0 to 11.0, or at approximately 5 K), these values have been regarded as standards for the overall oxidation potential of PS II.

In contrast, extremely high potential values for Phe *a* have been reported recently. A report by Rappaport et al. (2002) estimated the potential of *Synechocystis* sp. PCC 6803 (hereafter referred to as *Synechocystis*) to be in the −500 mV range (11). Kato et al., using a mutant of *Thermosynechococcus elongatus*, also supported a high value (−505 ± 6 mV at pH 6.5) under the presence of a stabilizer (1.0 M betaine) (13); this study used a sophisticated spectroelectrochemical method. The controversy over E_m(Phe *a*/Phe a^-) values indicates that the redox potential of Phe *a* in PS II is still under debate. Measurement of the E_m(Phe *a*/Phe a^-) value needs to be addressed more systematically, in order to fully understand the mechanism of water oxidation in PS II.

Cyanobacteria are frequently used to investigate the primary reactions in photosynthesis because of the ability to transform the cells and also because of the variability of pigment species they possess. *Acaryochloris marina* is a unique cyanobacterium which contains Chl *d* as the predominant pigment (more than 95%) and Chl *a* as a minor pigment (less than 5%) (14). The primary electron acceptor of PS II in this organism is Phe *a* (15, 16), on the contrary, assignment of the special pair is still controversial. We assigned Chl *d* dimer to the special pair based on results by absorption change and Fourier-transform Infra-red spectroscopy using highly purified samples (15), and other papers supported this view, which was examined using partly purified samples (17, 18). A different component (Chl *a* and Chl *d*

heterodimer) was also proposed for the special pair from work using partly purified samples (19, 20). Since the absorption maximum of Chl *d* is at longer wavelengths than Chl *a*, the energy gain by Chl *d* is lower than that of Chl *a* by approximately 0.08 V. This difference is significant for the reaction processes in PS II in *A. marina*. The high oxidation potential of the special pair is necessary for water oxidation. It is reported that the potential in *A. marina* is very similar to that of other cyanobacteria (21), however, experimental evidence for this interpretation is indirect; therefore, it is necessary to estimate the redox potential of Phe *a* in *A. marina*.

In this report, we estimated the potentials of Phe *a* under physiological conditions (pH 7.0, 25°C) in the presence of betaine (1 M) using samples of PS II complexes isolated from *Synechocystis* consisting of Chl *a* as the special pair, and from *A. marina* consisting of Chl *d* as the special pair. Spinach PS II complexes were used as a reference. We found a significant species-dependent difference in the redox potential of Phe *a* and in the effect of PS II stabilizers on the potential of Phe *a*. Based on these novel results, we discuss an energy diagram for electron transfer in PS II in oxygenic photosynthetic organisms.

Results

Properties of the samples

We examined the purities of the three samples by SDS-PAGE (Fig. 1). The polypeptide patterns of the samples were consistent with previous reports (15, 22, 23), indicating that the samples were highly purified. In the case of *A. marina*, a current purification step preceding the purification steps used in a previous report (15) almost completely removed CP43' (PcbC). The photoreduction of 2,6-dichlorophenol indophenol (DCIP) in the presence of diphenyl carbazide (DPC) was 1320 and 1710 μmole (mg Chl)$^{-1}$ hr^{-1} for *Synechocystis* and *A. marina*, respectively, indicating that the samples were suitable

for further study. We confirmed the absence of PS I components by fluorescence spectroscopy at −196°C (Fig. S1A) and Western blotting (Fig. S1D).

Phe *a* redox potential in *Synechocystis*

We examined the redox potential of Phe *a* at pH 7.0 in PS II complexes isolated from *Synechocystis* cells having a hexa-histidine tag at the C-terminus of the 47 kDa chlorophyll-protein (CP47). A light-*minus*-dark difference absorption spectrum exhibited characteristic peaks at 683 ± 0.3 (Fig. 2A), 451 ± 1.0, and 430 ± 1.0 nm (Fig. S2A), indicating that Phe *a* was reduced. In darkness the light-*minus*-dark absorption changes completely disappeared in a whole region of the difference spectrum, thus representing only reversible photoreduction of Phe *a*. We used the magnitude of the difference in absorption at 683 nm for titration, because the reproducibility and signal-to-noise ratio in the red region were much better than in the blue region (450 nm, which has been frequently used in previous work). We also observed a difference in the Qx region of Phe *a* at 543 nm; however, the magnitude of the difference was low (Fig. S2B). The reduction of Phe *a* was reversible in the range of redox potentials between −620 mV and −450 mV, as shown previously (8).

We estimated the standard redox potential, E_m(Phe *a*/Phe a^-), by plotting the observed potentials as a function of the relative fractions of reduced and oxidized forms of Phe *a*, i.e. [Phe *a*]/[Phe a^-] in a logarithmic scale, and obtained the E_m value as the y-intercept, −536 mV (Fig. 3A). The slope of the regression line was 64, slightly larger than the theoretical value of 59.2, indicating that the Nernst equation for a one electron transfer process was a good fit for these measurements. We estimated the deviation of this measurement to be ± 8 mV.

Phe *a* redox potential in *A. marina*

We measured the redox potential of Phe *a* in *A. marina* PS II core complexes. Since addition of a reducing agent, sodium dithionite, induces a blue shift in the absorption maximum of Chl *d* (24), it was difficult to monitor the absorption changes in the red region. Therefore, we measured changes in absorption maxima in the blue region (at 452 ± 1.0 nm, Fig. 2B). The E_m in the presence of betaine was estimated to be −478 ± 24 mV with a slope of 61 (Fig. 3B). This potential was significantly more positive than that for *Synechocystis*.

Comparison of spinach PS II with the two cyanobacterial species

For reference, we examined the potential of Phe *a* in PS II complexes isolated from spinach. We measured absorption difference spectra in both the red and blue regions. The resultant E_m redox potentials at pH 7.0 were estimated to be −532 ± 11 mV (Fig. 3C) and −523 ± 22 mV (Table 1) for detection at 680 nm and 450 nm, with a slope of 63 and 64, respectively. These data clearly indicate that the redox potential values did not depend on the monitoring wavelength. The redox potential values obtained in this study are summarized in Table 1.

Based on the above measurements, we concluded that difference in the redox potential of Phe *a* primarily depended on the difference in the special pair Chl, but not on the measuring conditions. Difference in the potential from the original report (−610 ± 30 mV) was mainly attributed to the effect of betaine.

The effect of betaine on redox potentials

We examined the effect of betaine (1.0 M) on the redox potential of Phe *a*, because this reagent is frequently used for recent measurements (13, 25) but not for the original report (6-8). In the case of *Synechocystis*, under the absence of betaine, the difference spectra in the red region (683 ± 0.3 nm) were essentially identical to those observed in the presence of

betaine (Fig. 2A vs. 2C). The E_m value for Phe *a* was −589 ± 11 mV (Fig. 4A), which was significantly more negative than in the presence of betaine. This estimate was close to the original report (−610 ± 30 mV), suggesting the betaine affected significantly the redox potentials of Phe *a*.

The effect of betaine was saturated at 1.0 M, since a higher concentration of betaine (1.2 M) did not further shift the potential (Fig. S3). Sucrose and mannitol, which are known stabilizers of PS II, also induced a shift in redox potential to approximately −530 mV (Fig. S3). The slopes for these measurements ranged from 57 to 61, and deviations were approximately ± 10 mV.

It was also the case of *A. marina*. In the absence of betaine, we observed a small red shift of the difference spectrum by 3 nm (Fig. 2B vs. 2D), and estimated the midpoint potential to be −544 mV (Fig. 4B) at two different wavelengths (a positive peak at 455 ± 1.0 nm, and a negative peak at 430 ± 1.0 nm, Fig. 2D) with deviations of 20 mV. This E_m value was significantly more positive than that of *Synechocystis*. These results clearly indicate that the effect of betaine was similar in *Synechocystis* and *A. marina*. The betaine-induced difference in the redox potential was larger in *A. marina* (ΔE_m = 66 mV) than that in *Synechocystis* (ΔE_m = 53 mV). This was the first indication on the effect of betaine on the redox potentials of Phe *a* and led to the complement of difference in the potential between our estimates and the original report.

Discussion

Midpoint potentials of Phe *a* from two cyanobacteria

We estimated the midpoint potential, E_m, of the primary electron acceptor of PS II under physiological conditions (pH 7.0, 25°C). In the originally published E_m measurements, non-physiological pH conditions were used (pH 8.0 to 11.0) (8). Such conditions may have

induced a pH-dependent shift in potential or denaturation of samples. However, our current conditions eliminated factors that hinder redox potential measurements, as shown by the reversibility of difference absorption spectrum of Phe *a* (Fig. 2).

The redox potential for Phe *a* in a dimethyl formamide solution was −620 to −640 mV (26, 27). On the contrary, we estimated the potentials of Phe *a* in PS II complexes to be −536 ± 8 mV for *Synechocystis* and −478 ± 24 mV for *A. marina* (Fig. 3). The value in *A. marina* was significantly higher than that in *Synechocystis*, and this estimate was confirmed by difference in the reduction of Phe *a* by addition of sodium dithionite. Phe *a* in PS II is not reduced by sodium dithionite, whose midpoint potential is −530 mV (28). However, we have already reported that Phe *a* in *A. marina* PS II is reduced by sodium dithionite (15), and that biochemical extraction of Q_A and Q_B induces a reduction in Phe *a*. These observations indicate that the *in vivo* potential of Phe *a* in *A. marina* PS II should be less negative than −530 mV. These observations were consistent with the measured potential of Phe *a* (−478 ± 24 mV). The difference in the estimated potentials of Phe *a* between the two cyanobacterial species is correlated to the energy differences in wavelengths of light absorbed by the different pigments.

Measurements from recent studies show deviations from our estimates. Rappaport et al. (2002) reported a value of approximately −500 mV on *Synechocystis* (11). Kato et al. used a mutant of *Thermosynechococcus elongatus*, in which *psbA1* and *psbA2*, genes encoding D1 protein, were deleted, and the glutamine residue at position 130 in the *psbA3* D1 gene was intrinsically coded in place of glutamic acid in *psbA1* (13). They added 1 M betaine to the reaction mixture and illuminated the samples in a rather long time (minimum 5 min), and estimated the potential to be −505 ± 6 mV at pH 6.5. Compared with our estimate (−536 ± 8 mV for *Synechocystis*), difference was not necessarily large, however their estimate cannot be straightforwardly compared with our data here or with the original report by

Klimov et al. (8).

As shown in this study, betaine induced a significant up-shift in redox potential (Fig. 4). If we calculate an up-shift of comparable magnitude (approximately 50 to 65 mV) for the *T. elongatus* mutant, the E_m potential would be approximately −555 to −570 mV in the absence of betaine. Compared with our results with *Synechocystis* (−589 ± 11 mV), this potential is still more positive by 20 to 35 mV. However, this difference is not unreasonable when a deviation of the measurements is considered. Furthermore, in Kato's experiment, a long-time illumination under a low redox condition might induce photoinhibition, which was suggested in the kinetics, and expression of un-constitutive *psbA3* gene may affect the redox potential, thus the estimation of redox potential by Kato et al. cannot be considered a standard value for Phe *a* in PS II. Examination of *Synechocystis* and/or spinach by a common method is required for comparison and comprehensive understanding.

Estimation of the redox potential of the special pair of PS II

It is critically important to estimate the redox potential of the special pair in PS II in the two cyanobacterial species for a comprehensive understanding of PS II even though the predominant Chls and the special pair Chls differ between the two species. An energy gain by light absorption in the two species was estimated to be 1.82 eV (P680) and 1.74 eV (P713) for *Synechocystis* and *A. marina*, respectively, and the energy difference between the excited state of the primary electron donor and Phe *a* was assumed to be 0.08 V (7), although measurements of this energy difference have not been consistent across studies (20, 21). In the case of *Synechocystis*, the redox potential of the special pair was estimated to be +1.20 V, and that for *A. marina* +1.18 V (Fig. 5). When a larger energy difference between the excited state of the special pair and Phe *a* was assumed (29), the estimated redox potential could shift to a more negative value by a maximum of 70 mV. Our estimations of the redox potential of

the special pair in the two cyanobacteria were very similar, which is consistent with a previous report showing no significant difference in the potential of the special pair in PS II across species (21). Our estimation does not provide direct evidence, but could indicate the levels of redox changes in the overall reactions in PS II. These values clearly indicate that the water oxidation system is conserved in terms of oxidation-reduction potentials, and suggest that the reaction components and reaction processes of water oxidation are also conserved.

Factors affecting the redox potential of Phe *a in vivo*

Structural analysis revealed that photoactive Phe *a* forms a hydrogen bond with the C9-keto group and D1-130 residue, which is glutamic acid in many oxygenic photosynthetic organisms including spinach, but is replaced by glutamine in *Synechocystis* and *A. marina* (30, 31). Replacement of glutamine with glutamic acid in a *Synechocystis* mutant induced an up-shift of potential by 15 mV (32). This tendency was reproduced in our measurements in the presence of betaine (−536 mV for *Synechocystis* and −532 mV for spinach). However, the shift does not seem to make a significant difference in the determination of the Phe *a* redox potential. These results clearly indicate that a hydrogen bond between Phe *a* and the D1-130 amino acid residue was not the primary determining factor of redox potential. Other factor(s) such as the surrounding environment, hydrophobicity, and dielectric constant, may be factors that significantly influence redox potential.

The effect of the Mn cluster on the Phe *a* potential may be indirect; however, it is important to consider the intactness of the Mn cluster under Phe *a* titration conditions. Upon titration, a reductant, such as sodium dithionite, is usually added to regulate the potential, and the oxygen-evolving activity is suppressed below 0 V (33). Based on these observations, we have not examined the intactness of the Mn cluster and related phenomena. It might also be the case of the reducing side of PS II; an electrostatic effect might also affect the potential of

Phe *a*. These points should be considered for a comprehensive understanding of Phe *a*.

Effect of betaine on the overall stability of PS II complexes

Betaine stabilizes PS II complexes under stress conditions such as dehydration (34), heat (35, 36), cold (37), and osmotic pressure (38). These effects may result from an overall stabilization of the structure of PS II, or, more directly, by binding of peripheral proteins needed for water oxidation to the PS II core complex (39). We clearly observed an up-shift in the midpoint potentials of Phe *a* in PS II complexes in the presence of betaine. Betaine did not enhance an initial rate of the DPC-DCIP photoreduction (Fig. S4A), nor enhanced an initial rate of oxygen-evolving activity (Fig. S4B). The polypeptide compositions of the two cyanobacterial PS II preparations are not identical: *A. marina* PS II core complexes do not contain any peripheral proteins. Even with this difference, the effect of betaine was evident, suggesting that betaine stabilizes the overall structure of PS II, which ultimately affects the potential of Phe *a* in the reducing site of PS II. Hydrophobic environment is necessary to stabilize PS II activity (40). For example, in the case of cyanobacterium *Anabaena cylindrica*, a water content less than 40 M is required, which is realized by a high concentration (1.8 M) of sucrose (40). It is also the case of *Anabaena variabilis*, in which 52 M water content is required. A similar effect might be realized when betaine is present in the mixture, thus the water environment caused by betaine might be regarded as a physiological condition in cyanobacteria, which might enforce a hydrophobic property in cytoplasm.

This study has characterized the following key characteristics of the redox potential of Phe *a*. (1) The potential of Phe *a* in the Chl *d*-dominated cyanobacterium *A. marina* was more positive (–478 ± 24 mV) than in *Synechocystis* (–536 ± 8 mV) in the presence of a stabilizer (1.0 M betaine). This was consistent with a lower gain of light energy by Chl *d*. (2)

The potentials of the primary electron donor of PS II are estimated to be approximately 1.20 V for both species, clearly indicating conservation in the properties of water oxidation systems in oxygenic photosynthetic organisms irrespective of the special pair chlorophylls. (3) Betaine induced a significant up-shift in the redox potential of Phe *a* in cyanobacteria (ΔE_m = 50 to 65 mV).

Materials and Methods

Preparation of samples

PS II complexes of *Synechocystis* were prepared by a procedure described earlier (41, 42). PS II core complexes from *Acaryochloris marina* MBIC 11017 were isolated as described previously (15), with slight modifications. Thylakoid membranes were isolated by mechanical disruption and differential centrifugation. PS II core complexes were solubilized in detergent (β-D-dodecyl maltoside, 1%, 4°C, dark). The first purification step was sucrose density gradient centrifugation, followed by fractionation using a UnoQ column. Finally, fractions containing purified PSII complexes were subjected to sucrose density gradient centrifugation. PS II complexes from spinach chloroplasts were isolated as reported previously (23).

The polypeptide composition of the purified samples was examined by SDS-PAGE, using a 16-22% separating gel with a 6% stacking gel. After electrophoresis, gels were stained with Coomasie Brilliant Blue R-250.

Absorption and fluorescence spectra were measured as reported previously (15). Fluorescence spectra were corrected for the spectral sensitivity of the detector. Chlorophyll concentration was estimated spetroscopically using the reported extinction coefficient (43, 44) after extraction of pigments with 80% acetone. DPC-DCIP photoreduction activity was measured as reported earlier (45).

Redox Titration

We used the previously determined titration procedures (6-8) with slight modifications. A sample was placed in a special glass cuvette with 3 ports. The redox potential of the medium in the cuvette was monitored at room temperature by means of a platinum electrode, with a calibrated Ag/AgCl (in saturated KCl) solution, and expressed relative to the normal standard hydrogen electrode. The electrode was calibrated with quinhydrone at pH 7.0 (Horiba, F-53, Japan).

We measured the redox potential of Phe *a* under physiological conditions (pH 7.0, 25°C), and used a low concentration of Chl (approximately 8 μg Chl ml^{-1}); this concentration was approximately 80-times lower than that adopted by Kato et al. (13). Experimental details for titration are given in *SI Materials and Methods*.

Determination of redox potentials was performed as follows. After obtaining data (minimum 20 data points for individual measurements), a baseline correction was applied. Subsequently, an electrode potential was plotted as a function of the relative fraction of oxidized and reduced forms of Phe *a* using a logarithmic scale, i.e. (log [oxi]/[red]) (see Fig. 3 and 4). Individual fractions were estimated by the difference in absorption in the red or blue region. A linear regression analysis was used to obtain the mid-point potential as the y-intercept and to estimate deviations from the regression line.

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Figure Legends

Fig. 1: SDS-PAGE of PS II complexes.

Lane 1, molecular weight markers; lane 2, spinach chloroplasts; lane 3, *Synechocystis*; lane 4, *A. marina.* CP47 (filled circles), CP43 (open circles), D2 (open squares), D1 (closed squares), and the cyt b_{559} α-subunit (open triangles) are indicated. Asterisk, the D1/D2 heterodimer. LMW, low molecular weight proteins.

Fig. 2: Light-minus-dark subtraction absorption spectra of PS II complexes at different redox potentials.

(A) *Synechocystis* monitored in the red region in the presence of betaine, (B) *A. marina* monitored in the blue region in the presence of betaine, (C) *Synechocystis* monitored in the red region in the absence of betaine, and (D) *A. marina* monitored in the blue region in the absence of betaine.

Fig. 3: Nernst plots of the titration of Phe *a* in PS II complexes of *Synechocystis*, *A. marina*, and spinach in the presence of betaine (1.0 M).

N, number of data points; r^2, correlation coefficient. Detection wavelengths are indicated in the upper right of each panel.

Fig. 4: Nernst plots of the titration of Phe *a* in PS II complexes of *Synechocystis* and *A. marina* in the absence of betaine.

N, number of data points; r^2, correlation coefficient. Detection wavelengths are indicated in the upper right of each panel.

Fig. 5: An energy diagram of PS II in two cyanobacteria species having different special pair Chls.

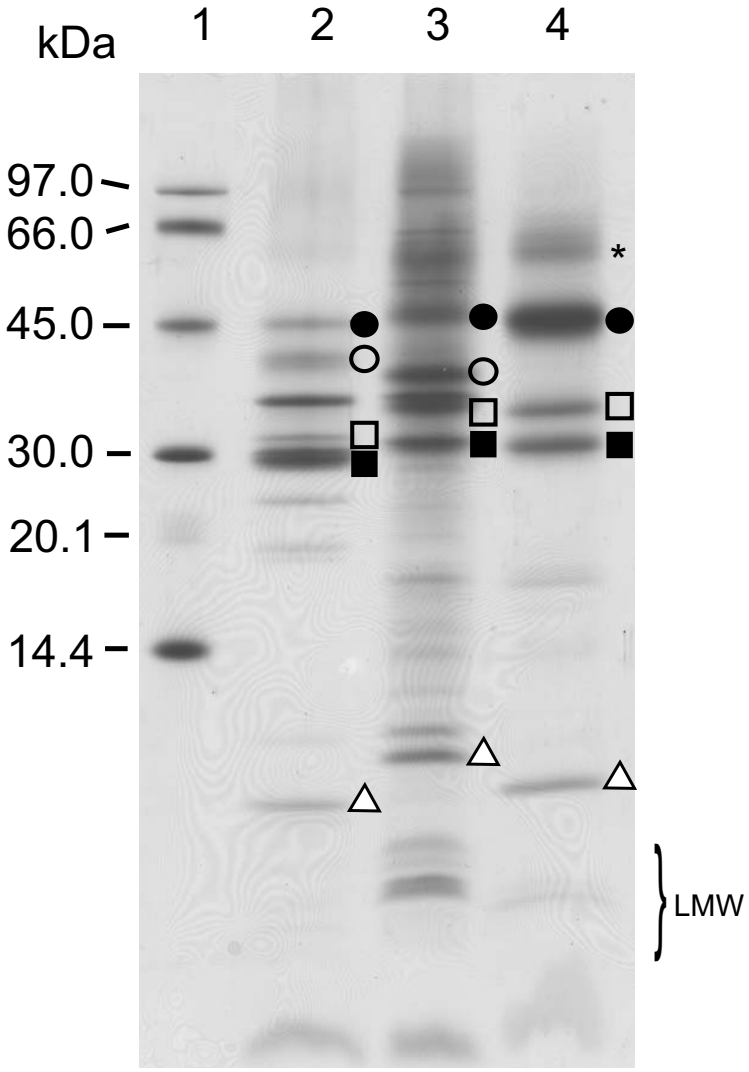
The difference in redox potentials between the excited state of the primary electron donor and Phe *a* are cited from (7).

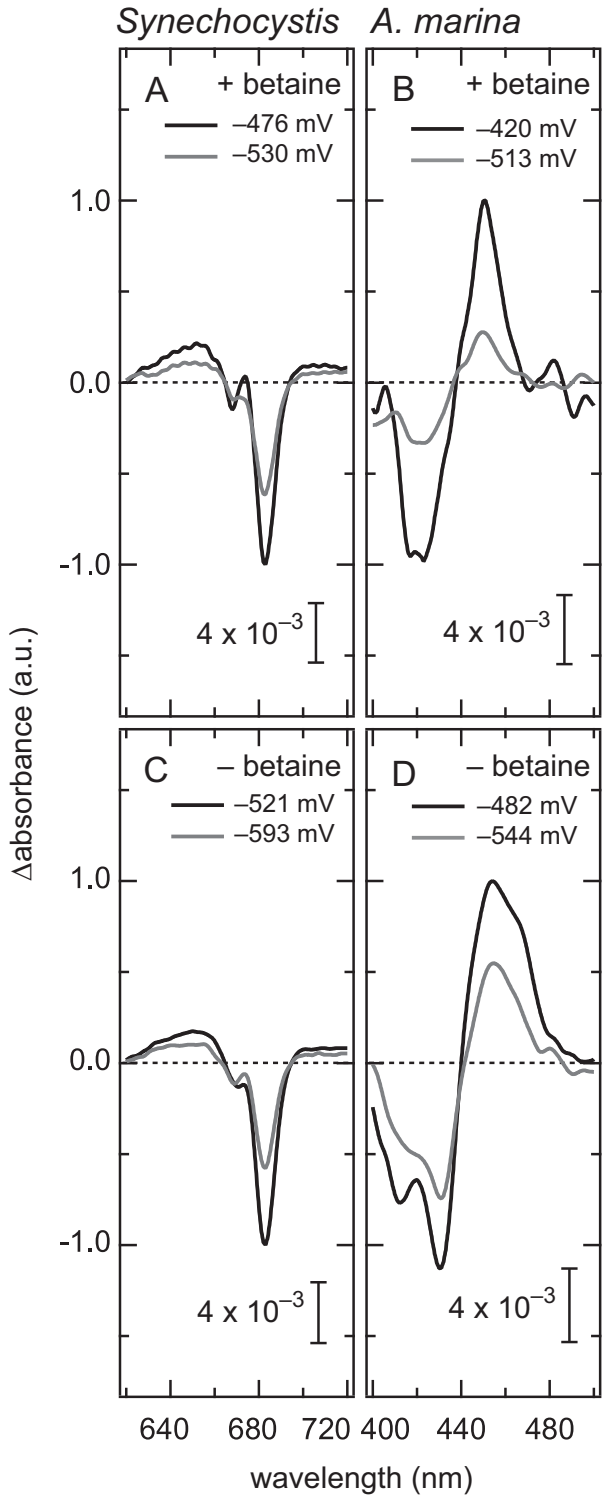
Table 1. Redox potentials of Phe *a* in PS II complexes at physiological pH

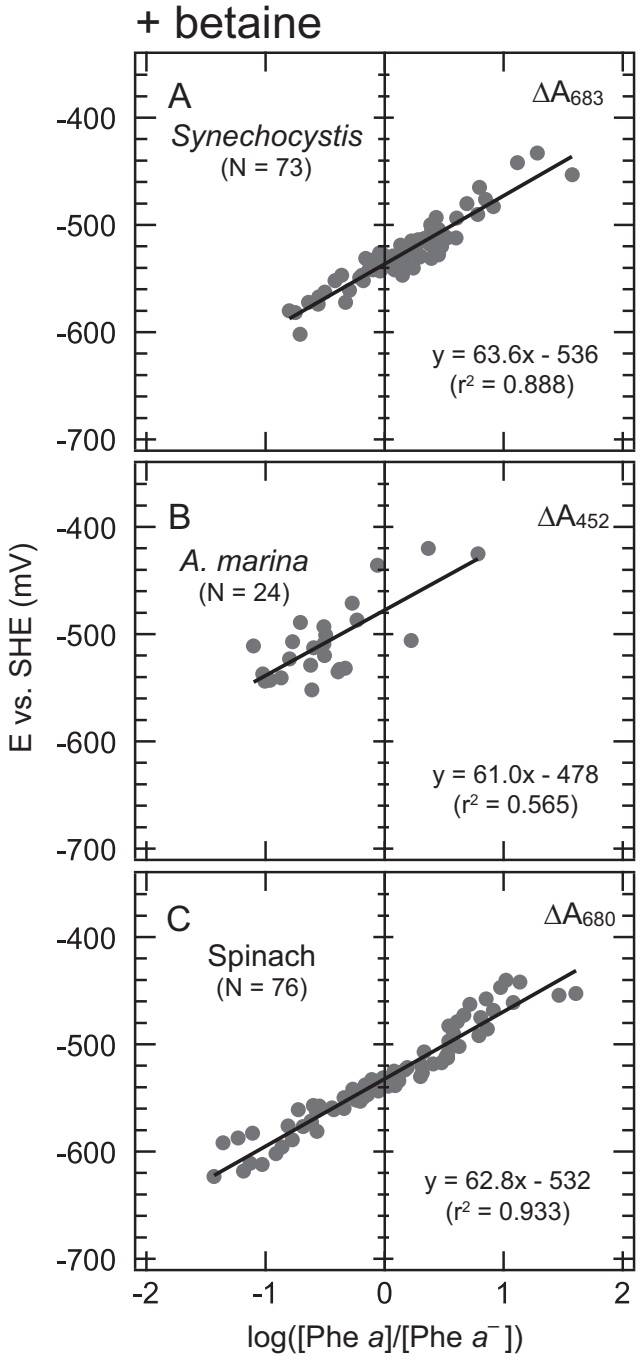
Samples	Additives	$E^{o'}$ (mV)	Slope	Detection wavelength (nm)
Synechocystis	+ betaine (1.0 M)	–536 ± 8	64	683
Synechocystis	+ betaine (1.2 M)	–532 ± 9	57	683
Synechocystis	+ sucrose (1.0 M)	–529 ± 8	59	683
Synechocystis	+ mannitol (0.5 M)	–528 ± 12	61	683
Synechocystis	– betaine	–589 ± 11	66	683
A. marina	+ betaine (1.0 M)	–478 ± 24	61	452
A. marina	– betaine	–544 ± 23	59	455
A. marina	– betaine	–544 ± 20	54	430
spinach	+ betaine (1.0 M)	–532 ± 11	63	680
spinach	+ betaine (1.0 M)	–523 ± 22	64	450
(pea, spinach	– betaine	–610 ± 30		685)*

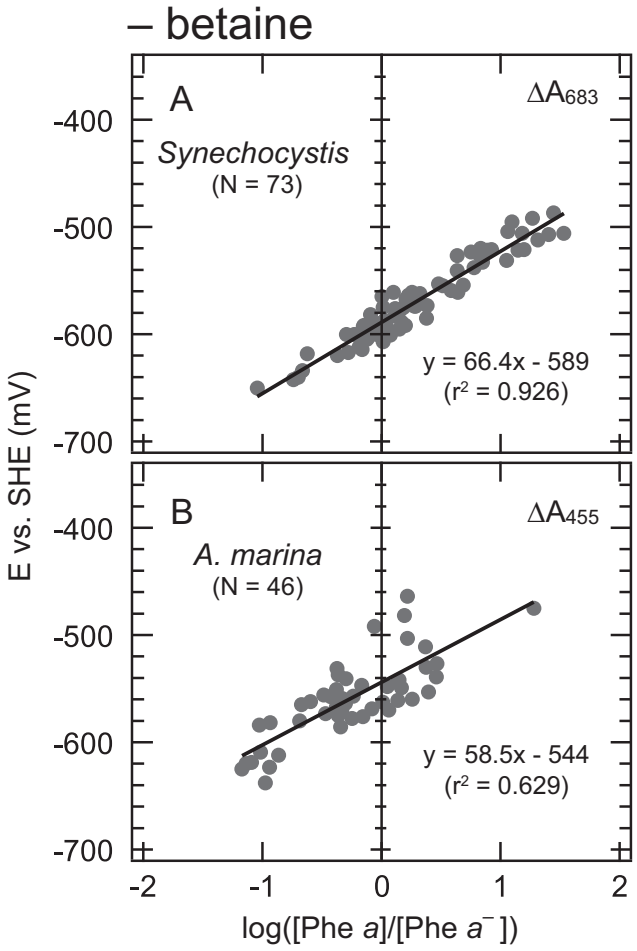
*by Klimov et al. (1979)

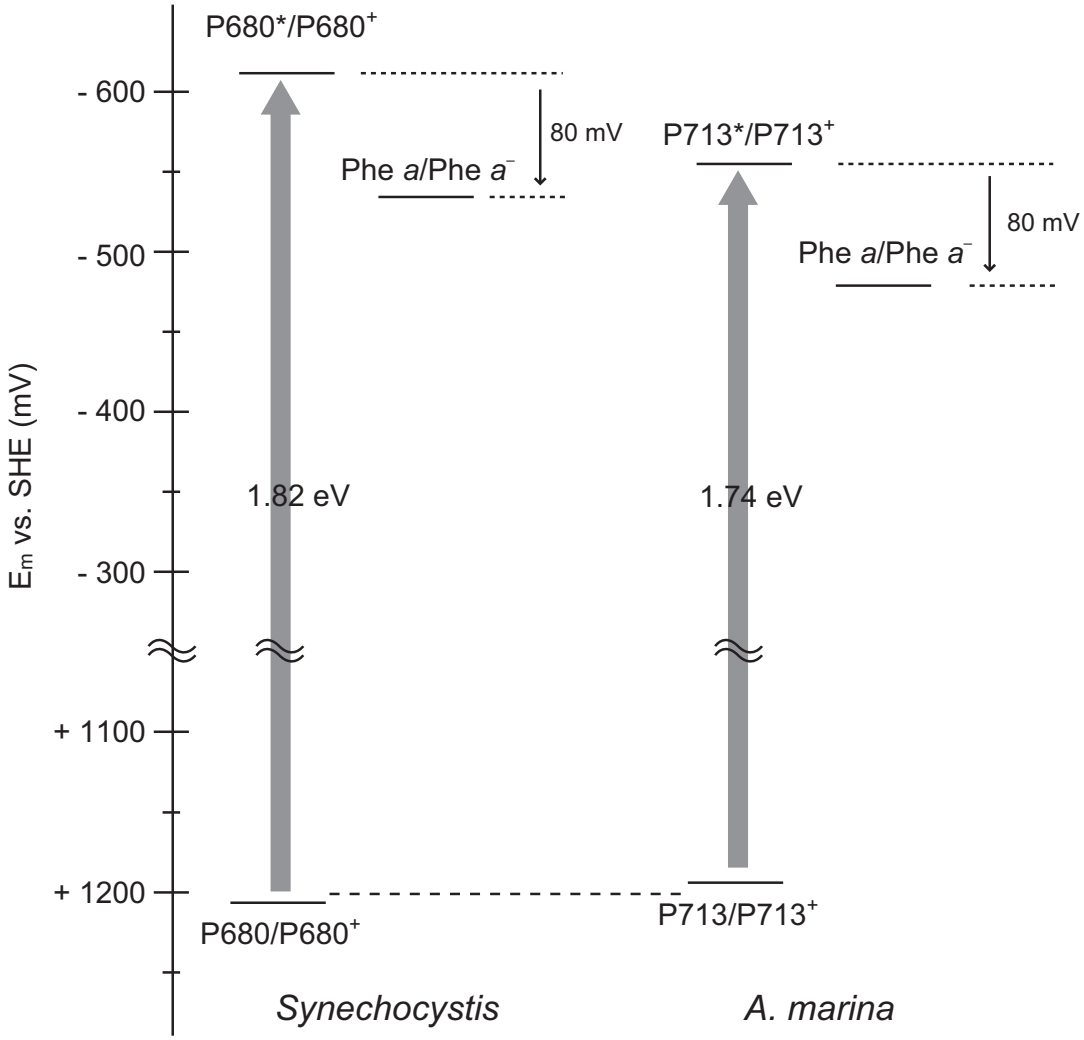
A




Synechocystis
A. marina
A
+ betaine
–476 mV
–530 mV
B
+ betaine
–420 mV
–513 mV
C
– betaine
–521 mV
–593 mV
D
– betaine
–482 mV
–544 mV
1.0
0.0
-1.0
4 x 10–3
Δabsorbance (a.u.)
640
680
720
400
440
480
wavelength (nm)


+ betaine
A
ΔA683
Synechocystis
(N = 73)
y = 63.6x - 536
(r2 = 0.888)
B
ΔA452
A. marina
(N = 24)
y = 61.0x - 478
(r2 = 0.565)
C
ΔA680
Spinach
(N = 76)
y = 62.8x - 532
(r2 = 0.933)
E vs. SHE (mV)
-400
-500
-600
-700
-2
-1
0
1
2
log([Phe a]/[Phe a−])


– betaine
A
ΔA683
Synechocystis
(N = 73)
y = 66.4x - 589
(r2 = 0.926)
B
ΔA455
A. marina
(N = 46)
y = 58.5x - 544
(r2 = 0.629)
E vs. SHE (mV)
-400
-500
-600
-700
-2
-1
0
1
2
log([Phe a]/[Phe a−])


P680*/P680+
- 600
- 500
- 400
- 300
+ 1100
+ 1200
Em vs. SHE (mV)
80 mV
Phe a/Phe a−
1.82 eV
P680/P680+
P713*/P713+
80 mV
Phe a/Phe a−
1.74 eV
P713/P713+
Synechocystis
A. marina