Spectroelectrochemical Determination of the Redox Potential of P700 in Spinach with an Optically Transparent Thin-layer Electrode

Akimasa Nakamura,* Tomoyuki Suzawa, and Tadashi Watanabe
Institute of Industrial Science, The University of Tokyo, 4-6-1, Komaba, Meguro-ku, Tokyo 153-8505

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Optimization of experimental conditions for spectroelectrochemistry with an optically transparent thin-layer electrode allowed us to determine the redox potential of spinach P700, the primary electron donor of photosystem I, to be +469 mV vs. SHE with significantly high reproducibility (±2 mV for 12 independent samples).

One of the salient features of photosynthesis is its ultimate quantum yield, namely ca. 1.0 after many intermolecular electron transfer steps. This should be supported by delicate redox potential tuning among the molecules involved. Though clarification of this aspect would lead to a better understanding of the photosynthetic mechanism, the redox potentials of electron transfer components still remain ambiguous. In view of this, we have attempted in this work, as a first step, to measure the redox potential of spinach P700, the primary electron donor of photosystem (PS) I.

The redox potential of P700, reported in past four decades, ranges from +360 mV to +520 mV vs. SHE.[1] Such scattering of as much as 160 mV may have resulted partly from limited accuracy of chemical redox titration used in most works. To unravel the cause(s) for the scattering in previous reports, it is desirable to strictly control the potential by an electrochemical means and examine the effects of such factors, that can affect the P700 redox potential, as the nature of detergent and its concentration used for sample preparation. For this purpose, spectroelectrochemistry in the presence of redox mediators is useful and was employed for redox potential measurement of P700 in one occasion.[2] However, the previous study was done with an ultra thin-layer electrode (10–15 μm optical path length) for IR absorption spectra measurement, and hence required a very high sample concentration, ca. 160 mM Chl *a*. At this concentration, the PS I sample should tend to be a viscous pellet-like solution, that renders sample handling difficult. The high viscosity of the sample and a very small cell thickness also potentially bring ambiguities on the P700 redox potential due to a higher solution resistance. Here we developed spectroelectrochemical conditions that readily allow determination of the P700 redox potential at a Chl concentration much lower than the previous study, with a particular attention to the efficiency of redox mediators in the P700 electrochemistry.

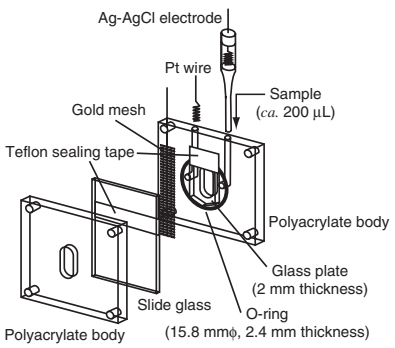

Figure 1. Schematic representation of the OTTLE cell.

The experiments were done with a laboratory-designed OTTLE cell (Figure 1). A gold mesh working electrode modified with 4,4′-dithiodipyridine (Aldrich)[3] was sandwiched between a glass plate surrounded by an O-ring and a slide glass, and then fixed with a pair of polyacrylate plates. The optical thickness determined by coulometry was 180 μm. The reference and counter electrodes were an Ag–AgCl (sat. KCl) electrode and a platinum wire, respectively. The electrode potential is hereafter reported against SHE. The formal potential of $K_3Fe(CN)_6$ determined with the OTTLE cell by spectroelectrochemistry and cyclic voltammetry agreed well with the reported value.[4] PS I was prepared from spinach as described previously[5] and suspended with 0.5% dodecyl-β-D-maltoside, 50 mM Tris-HCl (pH = 8.0), 0.2 M KCl, and redox mediators described below. The Chl *a* concentration was typically 1.5–2 mM, corresponding to 8–12 μM of P700.

The accuracy of redox potential measurement by spectroelectrochemistry heavily depends on how to find the redox equilibrium state clearly. For P700, the redox-induced absorbance change under these conditions was on the order of 0.001–0.01. To find the redox equilibrium of P700 clearly from such small absorbance changes, the latter must reach a steady state as rapidly as possible after potential application. Thus, the choice of redox mediators that facilitate rapid redox equilibration is essential for precise determination of the P700 redox potential. How-

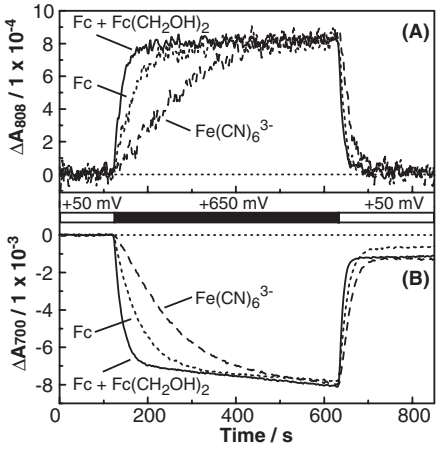

Figure 2. Time courses of absorbance changes at 808 nm and 700 nm during potential steps in the presence of 1 mM $K_3Fe(CN)_6$, 100 μM Fc, and 50 μM Fc + 50 μM Fc-dimethanol.

ever, the efficiency of mediators in the redox reaction of P700 has not been studied in detail. In most of previous chemical titrations, the $Fe(CN)_6^{3-/4-}$ couple was used as mediator and for adjusting the ambient redox potential.[6] However, in the OTTLE experiment, $Fe(CN)_6^{3-}$ ($E^{\circ\prime} = +433$ mV) did not act as an efficient mediator for P700 oxidation. When P700 was reduced at $E = +50$ mV and then oxidized at $E = +650$ mV in the presence of 1 mM $Fe(CN)_6^{3-}$, the positive absorbance at 808 nm, where only $P700^+$ exhibits absorption,[5] gradually increased and reached a steady state in as long as 8 min (Figure 2A). In contrast, ferrocene (Fc; $E^{\circ\prime} = +418$ mV) worked as a more efficient mediator, even when its concentration was tenth of $Fe(CN)_6^{3-}$ (Figure 2A). Addition of 1,1′-Fc-dimethanol ($E^{\circ\prime} = +476$ mV) to Fc further accelerated P700 oxidation and reached a steady state in less than 2 min (Figure 2A). When the electrode potential was returned from +650 to +50 mV, the ΔA_{808} value fell into the level before application of the anodic potential, indicating a fully reversible nature of the P700 redox reaction. This behavior remained the same over 30 cycles of potential journey. Based on these results, 50 μM Fc + 50 μM Fc-dimethanol was chosen as mediators in what follows.

The significant difference in P700 oxidation between Fc and $Fe(CN)_6^{3-}$, despite their similar formal potentials, would be due in part to their different accessibility to the vicinity of P700. PS I is negatively charged at pH = 8 because of its acidic nature (pI ≈ 4) and hence $Fe(CN)_6^{3-}$ is less likely to reach the PS I core than positively charged ferricinium ion.

The differential extinction coefficient of P700 is about 9-fold larger at 700 nm than that at 808 nm.[5] When the redox reaction was monitored at 700 nm, the initial phases of P700 oxidation were identical to that at 808 nm. However, the ΔA_{700} value tended to decrease even after ΔA_{808} reached a steady state, and ΔA_{700} was partially irreversible after reduction of $P700^+$ at $E = +50$ mV (Figure 2B). The continuous decrease and partial irreversibility of ΔA_{700} is due to irreversible oxidation of bulk antenna Chl *a* other than P700, as is evidenced by a bleaching of Chl *a* after redox cycling (data not shown). Thus, the time course measurement of ΔA_{700}, employed for monitoring the redox-induced absorbance changes of P700 in the previous study,[2] is not adequate to accurately determine the P700 redox potential, in particular for PS I accompanied by a larger amount of antenna Chl molecules in higher plants and green algae.[5]

By monitoring the ΔA_{808} values in redox cycles (Figure 3), the Nernstian plots were constructed (Figure 4). The ΔA_{808} plot gave a slope of 60.7 ± 1.7 mV/decade (number of independent samples (N) = 12), reflecting a reversible one-electron reaction, and the P700 redox potential of +469 ± 2 mV ($N = 12$). The high reproducibility of the P700 redox potential, ±2 mV, for twelve independent samples, is far superior to that by chemical titration where more than ±10 mV of deviation is generally observed.

The excellent reproducibility of the P700 redox potential in the OTTLE experiments as compared with previous chemical titration, does not come only from the strict control of electrode potential but also from careful determination of the redox equilibrium state, that was made possible by selecting Fc and Fc-dimethanol as mediators and continuous monitoring of absorbance change at 808 nm. Chemical titration with a less efficient mediator $Fe(CN)_6^{3-/4-}$ and monitoring of the P700 redox state by a series of flash-induced absorbance change measurements[6] could bring considerable ambiguities in finding the redox equilibrium state after changing the ambient redox potential. This might be one of the reasons for the heavy scattering of the P700 redox potential reported previously.

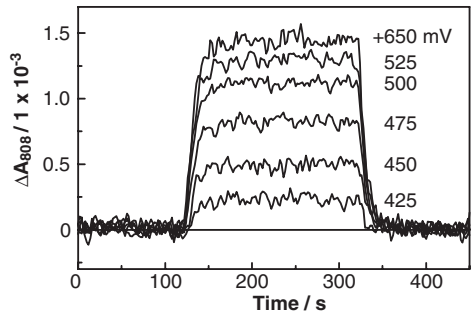

Figure 3. Time courses of ΔA_{808} during P700 oxidation at varying electrode potential and P700 reduction at $E = +50$ mV.

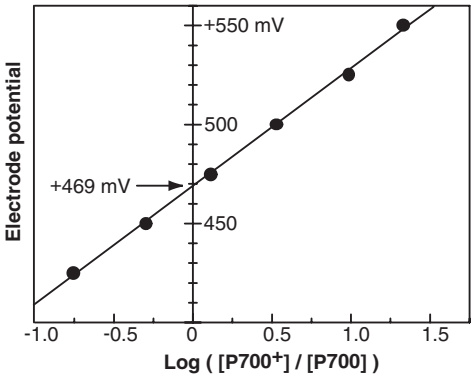

Figure 4. Nernstian plots for P700 based on ΔA_{808} values in Figure 3. The ΔA_{808} values are determined after smoothing of the traces in Figure 3 by simple moving average method.

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