Probing into the Redox Potential Tuning in Photosynthetic Machinery

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Photosynthesis enjoys an ultimate quantum yield, being *ca*. 1.0 after many energy and electron transfer steps, which undoubtedly is backed up by an exquisite redox potential tuning among the molecular components involved. This aspect, however, is still far from being unraveled. We have attempted here, as a first step, to spectroelectrochemically determine the *in vivo* redox potential *E*°' of P700, the primary electron donor of photosystem (PS) I, for which the reported *E*°' values exhibit a significant scatter (from +360 to +520 mV vs. SHE) due primarily to the use of chemical redox titration and/or to a possible species-dependence of the P700 potential.

The experiments were done with a laboratory-designed optically transparent thin-layer cell, in which a gold mesh working electrode modified with 4,4'-dithiopyridine was sandwiched between a pair of glass plates. The optical thickness was 180 μm, and the cell accommodates less than 200 μL of sample solution. PS I was prepared from various organisms as described elsewhere,[1)] and suspended with 0.5% dodecyl-β-D-maltoside, 50 mM Tris-HCl (pH 8.0), 0.2 M KCl, and redox mediators (50 μM ferrocene + 50 μM 1,1'-ferrocene-dimethanol, a combination found optimal after a systematic study).

On application of a positive potential to the working electrode, the visible spectrum developed an absorbance dip at 700 nm and a broad absorbance gain centered at around 808 nm.[1)] Since the former (ΔA_{700}) is interfered with the irreversible spectral change of bulk antenna molecules, we monitored ΔA_{808} throughout. During a potential journey, the ΔA_{808} value exhibited a completely reversible behavior as seen in **Fig. 1**.

A nernstian plot of such data yielded a nicely straight log([P700$^+$]/[P700]) versus potential curve (**Fig. 2**), from which the *E*°' value of P700 was determined. By twelve independent runs on spinach P700, for instance, the *E*°' value was found to be +469 mV *vs*. SHE with a sufficiently small error margin (± 2 mV) and a nernstian slope of 60.7 ± 1.7 mV/decade.[2)]

Similar measurements were conducted on various oxygenic photosynthetic organisms, and the results are summarized in **Fig. 3**. It is clearly seen that the P700 redox potential possesses a significant variation, far beyond the experimental error range (± 2 mV), among the species. This unprecedented finding would deserve further investigation, incorporating *E*°' measurements of electron donors/acceptors around P700, for a better understanding of the molecular mechanism/machinery of photosynthesis.

References
1) A. Nakamura, M. Akai, E. Yoshida, T. Taki, and T. Watanabe, *Eur. J. Biochem*., **270**, 2446-2458 (2003).
2) A. Nakamura, T. Suzawa, and T. Watanabe, *Chem. Lett*., **33**, No.6, in press (2004).

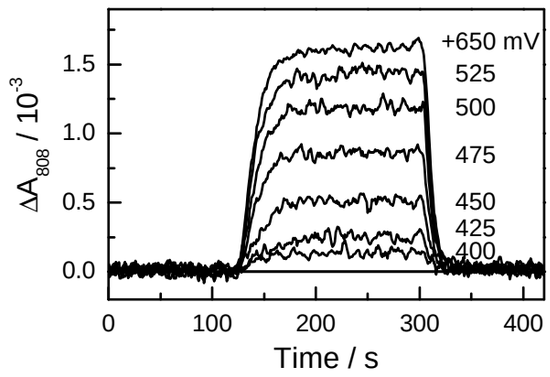

Fig. 1. Time courses of ΔA_{808} by P700 oxidation at varying electrode potential and re-reduction at +50 mV.

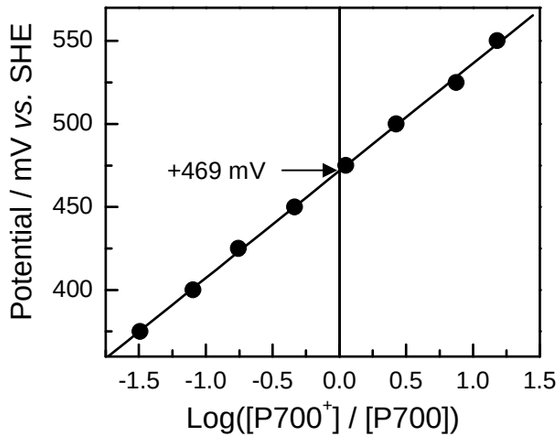

Fig. 2. Nernstian plots for P700 based on ΔA_{808} values in Fig. 1.

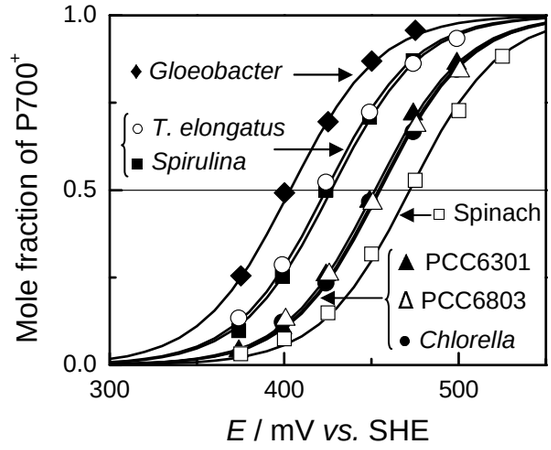

Fig. 3. Nernstian plots for P700 prepared from a series of plant species.