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Primary processes in photosystem I reaction center at cryogenic temperatures

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Introduction

The structural model at an atomic resolution has been reported for the photosystem I (PS I) RC, where there are 6 chlorophyll molecules (Chl) in the electron transfer (ET) system. It has been, however, difficult to detect the elementary processes in the ET system. This is partly due to the large number (about 90 Chls) of antenna chlorophylls that surround the ET system. Extraction of most of the antenna chlorophylls by diethyl ether provides us a unique opportunity to study the elementary steps of the primary ET in the PSI RC of higher plant [Ikegami et al., Kumazaki et al.].

The primary ET in purple bacterial RC shows unique temperature dependence. The ET from the excited state of the special pair of bacteriochlorophylls to the acceptor bacteriopheophytin becomes faster at lower temperatures. This seems to be explained by the ET occurring from the lowest vibrational level of the initial potential surface [Zinth et al.].

How is this feature common to other photosynthetic RCs? This question is here addressed with the help of PSI RC from spinach that has about 12 – 13 Chls/P700 (P700-enriched RC).

Materials and methods

The P700-enriched RC particles were prepared as previously reported [Ikegami et al.]. Sodium dithionite was added to final concentrations of 10 mM at pH 10, in order to prepare the RC particles under the P700-neutral conditions. The RC particles under the P700-preoxidized conditions were prepared on addition of 1 mM ferricyanide. These solutions were further diluted by glycerol to a glycerol: water ratio of 6:4 (v: v). The sample was filled into an acrylic cuvette with an optical path length of 2 mm. The cuvette was set in a cryostat, which slowly cooled the cuvette to a desired temperature (e.g., 4 hours until 9 K). All the procedures after the dilution by glycerol were done under dim light. For the P700-neutral sample at 280 K, 20 mM phosphate buffer (pH 8) without glycerol was used as the solvent. The RCs were reduced by ascorbate and 2,6-dichlorophenolindophenol with the final concentrations of 7 mM and 70 μ M, respectively.

The transient absorbance changes were measured as the difference of the transmission between pump-on and pump-off conditions [Kumazaki et al.]. The pump and probe pulses came from an amplified 1 kHz femtosecond laser. Excitation wavelength was 630 nm. Femtosecond continuum pulses were used as the probe. The pump pulses were chopped at 5 Hz, and the probe spectra were synchronously recorded at 10 Hz. The instrument-response

function (0.25 ps full width at half maximum) and the wavelength dependence of the delay zero were determined by optical Kerr effect cross correlation between the pump and probe pulses. The angle between the pump and probe polarizations was set at the magic angle (54.7°). A home-built translational optics introduced the pump and probe beams to the sample in the cryostat. In this scheme, the sample position was fixed but the beam-overlapping region in the sample was continuously changed, while the probe beam was constantly incident on photodiode array attached to an exit port of a monochromator. This enabled us to minimize artifacts from long-lived transients. Unavoidable long-lived transients that appear as transient absorbance changes at negative pump-probe delays were subtracted from all the data presented below.

Results

Transient absorption spectra at 1 ps and 110 ps are shown in Fig. 1. Under the P700-preoxidized conditions, decay of excited chlorophylls is monitored. The area of the negative absorbance changes (photobleaching plus stimulated emission (PB/SE)) between 660 and 710 nm largely represents the number of excited RCs. About 90 % of the area at 1 ps decays within 110 ps, at temperatures of 9, 77 and 280 K (spectra at 77 K are not shown). This indicates that 90 % of the excited chlorophylls are relaxed to their ground states by the excitation transfer to P700⁺ and internal conversion at P700⁺.

Under the P700-neutral conditions, there remain more PB/SE signals at 110 ps than those under the P700-preoxidized conditions. There are also positive absorbance changes at 110 ps in the 720 – 770 nm region, which represents the radical pair of chlorophylls. Since the electron acceptor phylloquinone is removed from the P700-enriched RC, the chlorophyll radical-pair state should be accumulated on a picosecond time scale. The ratios of the areas of the PB/SE signals between 1 and 110 ps are similar at all the measured temperatures. For a comparison, the transient spectra under the P700-neutral conditions are scaled so that the average amplitude between 695 and 705 nm is –0.008 at 110 ps. Given this scaling, there is a noticeable temperature dependence of the absorbance change around 745 nm. More radical-pair state at 110 ps is observed at 280 K than at 9 K.

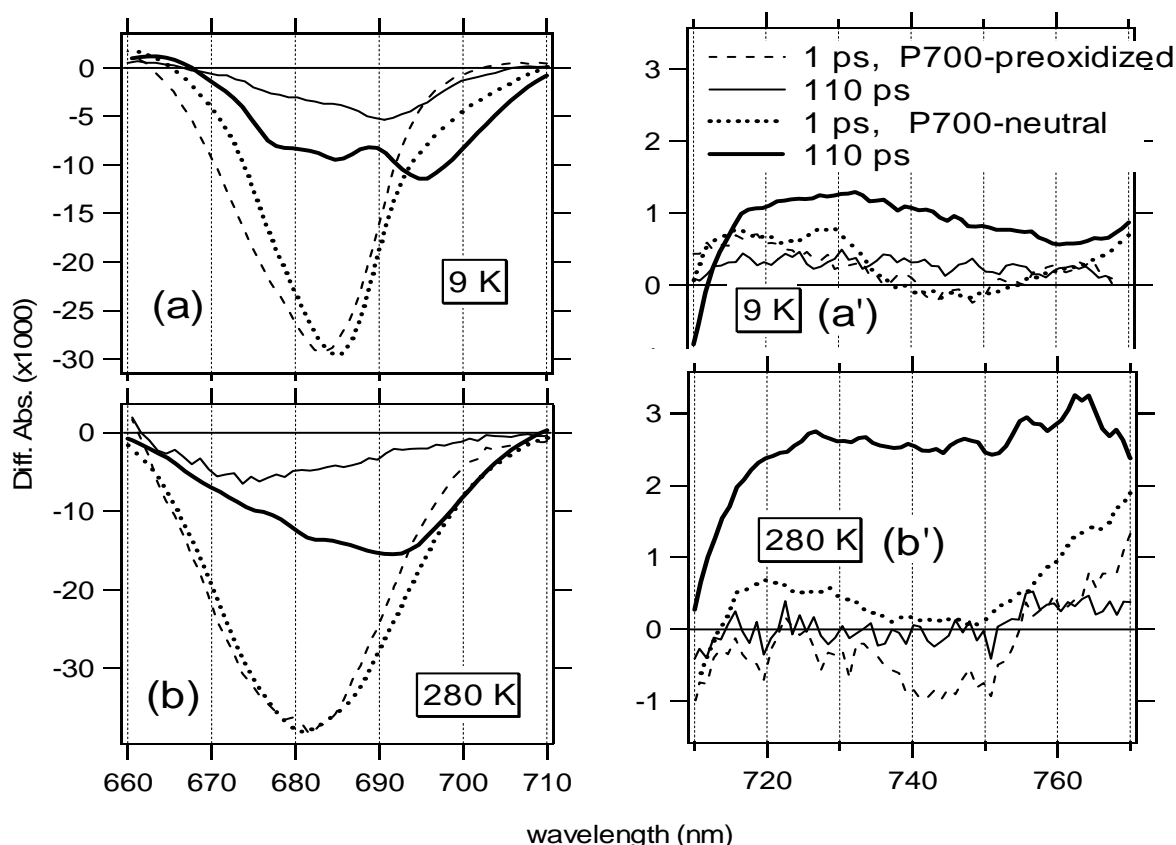


Fig. 1. Transient absorption spectra of the P700-enriched RC upon the 630 nm excitation. Absorbance of the samples were 0.8(\pm 0.1)/2mm.

The transient absorption kinetics probed at 700(\pm 5) nm and 745(\pm 10) nm under the P700-neutral conditions are shown in Fig. 2. The 745 nm kinetics demonstrate that the final extent of the primary charge separation is larger at higher temperature. Only about 40 % of the RCs with their P700 excited show the primary ET at 9 K, compared with the same reaction at 280 K. Extended scans of the delay time up to 1 ns have confirmed that there is no further increase of the radical-pair state (data not shown).

Discussion

Since the excited state of P700 shows an absorption at around 745 nm [Kumazaki et al.], the long-lived transient observed at 9 K may contain a contribution by P700*. We thus know only a higher limit (about 40 %) for the ratio of the RC that show the primary ET at 9 K. Some RCs in the P700-enriched RC at 10 K, however, do show charge recombination reaction, which is a result of the primary charge separation [Ikegami et al.]. It is thus suggested that the primary photochemistry of the P700-enriched RC at low temperature is highly heterogeneous. Some portion of the RC (\leq 40 %) shows the charge separation on a picosecond time scale even at around 9 K, but the other RC (\geq 60 %) does not show charge separation at 9 K.

The rate constant of the primary ET from P700* in the P700-enriched RC is estimated to be $(0.5 - 0.8 \text{ ps})^{-1}$ at a physiological temperature [Kumazaki et al.], which is in good agreement with those predicted for more intact PSI RCs retaining about 100 Chls/P700 [Karapetyan et al.]. To our knowledge, however, there has been no report on the temperature dependence of the rate and/or efficiency of the primary ET from P700* in PSI RC with about 100 Chls. In

such PSI RCs, relatively high quantum yields of the fluorescence at low temperatures seems to be explained not by the change in the ET, but by the trapping of excitation by the long-wavelength-absorbing chlorophylls with their absorption peaks even redder than that of P700.

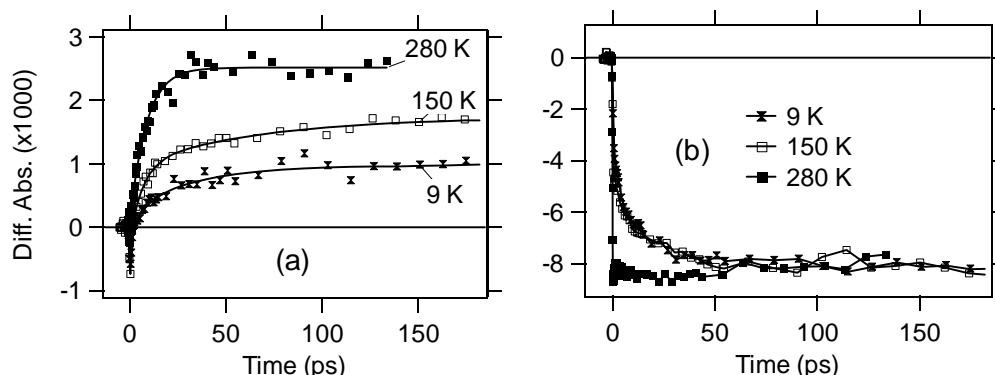


Fig. 2 Transient absorption kinetics probed at 745 (+/-10) nm (a), and at 700 (+/-5) nm (b). Excitation wavelength is 630 nm. Absorbance of the samples were 0.8(+/-0.1)/2mm.

In case that the drop of the quantum yield is a special feature of the P700-enriched RC and not observable in intact PSI RC, how would the difference arise? P700-enriched RC shows a quantum yield of the primary ET of 95 % at 290 K. The primary ET may appear to be highly heterogeneous only at low temperatures in the following way. There are two ET pathways: one requires thermal activation, but the other does not. If one Chl in the activationless ET pathway is absent in some fraction of RCs, there is no primary ET at low temperatures in this fraction of RCs.

Acknowledgments

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