

FEMTOSECOND SPECTROSCOPY OF PSII REACTION CENTERS: NEW RESULTS

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1. Introduction

The water-oxidation process of photosynthesis is driven by the conversion of light energy into chemical potential in the photosystem II (PSII) reaction center (RC) where rapid energy transfer to the primary electron donor, P680, and charge separation between P680 and the active pheophytin (Pheo) occur. We have reexamined these processes in isolated spinach PSII RCs using femtosecond pump-probe methods at lower pump energy and higher S/N than our previous studies (1,2). Furthermore, we have pumped directly into both the high and low energy sides of the 676-nm absorption band of the RC while probing at 544 nm, where the disappearance of the Pheo ground state can be monitored.

2. Procedure

PSII RCs (6 Chl/2 Pheo) were isolated from market spinach by standard techniques (3). Samples were run at 5°C under anaerobic conditions using an enzymatic oxygen-scrubbing system (4). Pheo was phototrapped in the presence of freshly made 1.5-mM dithionite. Time-resolved pump-probe kinetic spectroscopy was carried out with 180-fs time resolution using a Ti-sapphire laser-driven optical parametric amplifier (5) with the pump laser polarized at the magic angle relative to the polarized probe beam. The spectral width of the excitation pulses was about 7 nm (FWHM). Pump energies were 50 nJ to 1 μJ, and the spot size was 250 μm. The S/N levels represented by these data are around 50-60, and the analyses reported are averages of fits of individual data sets at 544 nm (i.e., not global analyses).

3. Results and Discussion

Time evolution of transient absorption changes excited by pumping PSII RCs at 683 nm (where P680 absorbs) or 665 nm (where RC antenna pigments absorb) and probing at 544 nm (Fig. 1 inset) elicited a rapid increase in absorption within the time of the pump pulse, and, subsequently, three distinct kinetic components representing decreases in absorption:

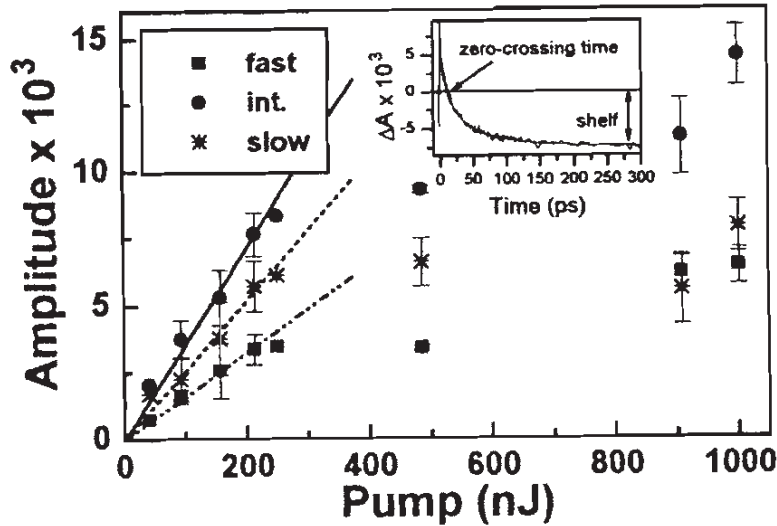


Figure 1. Amplitudes of the three kinetic components vs. pump energy. The inset shows a typical kinetic trace. Pump, 683 nm; probe, 544 nm.

a fast component with τ s on the order of 1-3 ps, an intermediate one with τ s of 10-25 ps, and a slow one with τ s ranging from 50-100 ps. The amplitudes of the three bleach components versus pump energy are linear up to 250 nJ at both pump wavelengths (Fig. 1 shows 683 nm data). Between 250 nJ and 1 μ J the amplitudes continue to increase but at a lesser slope. Zero-crossing times (ZCT, defined in Fig. 1 inset) increase with increasing pump energy from a minimum of about 10 ps at 683 nm excitation and 20 ps at 665 nm excitation. ZCTs provide a convenient way of comparing from laboratory to laboratory the actual pump intensity "seen" by a sample. Whereas the amplitudes of the three kinetic components increase with pump energy, there does not seem to be a consistent change in the relative percentage of the three components over the pump energy range investigated (data not shown). However, the percentage of intermediate time component appears greater, and the lifetimes of all three components increase somewhat when pumping at 665 nm vs. 683 nm.

To further examine this point, we obtained transient absorption spectra of PSII RCs at 500 ps and 1 ps after low energy pump pulses at 665 nm and 683 nm. Figure 2A shows that at 500 ps, transient absorption spectra in the 500-nm to 600-nm range are independent of pump wavelength. This indicates that energy transfer from antenna pigments, absorbing at 665 nm, to P680 is close to 100% efficient. On the other hand, Figure 2B shows that at short time there is a substantial difference in the spectra generated by the two pump wavelengths. The 665 nm minus 683 nm difference spectrum at 1 ps is rather featureless, and the species it represents is probably a Chl pigment excited state that disappears with a τ of about 29 ps. These data provide information about the "donor" state that transfers energy to P680 and also explains why more intermediate component is generated by 665-nm vs. 683-nm pump light on a relative basis, as mentioned above.

In an attempt to determine which of the three kinetic components is associated with primary charge separation, we reduced the RCs by phototrapping Pheo⁻ in the presence of dithionite. Figure 3 shows that under our conditions, we pre-reduced about half of the active Pheo on average over the time period that the spectra were obtained. The negative "shelf"

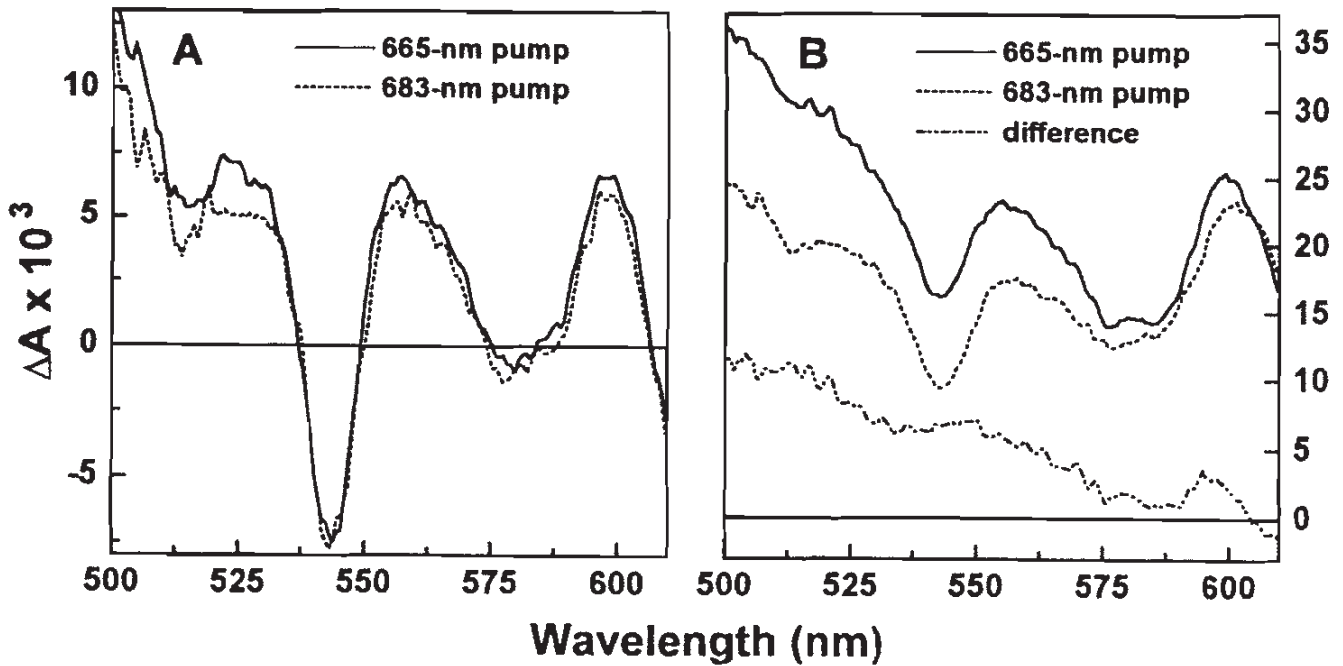


Figure 2. Transient absorption spectra at 500 ps (A) or 1 ps (B) after 665 nm or 683 nm pump pulses. The absorption of PSII RCs are approximately equal at these two wavelengths, and the pump energy of the flashes were also the same (~250 nJ).

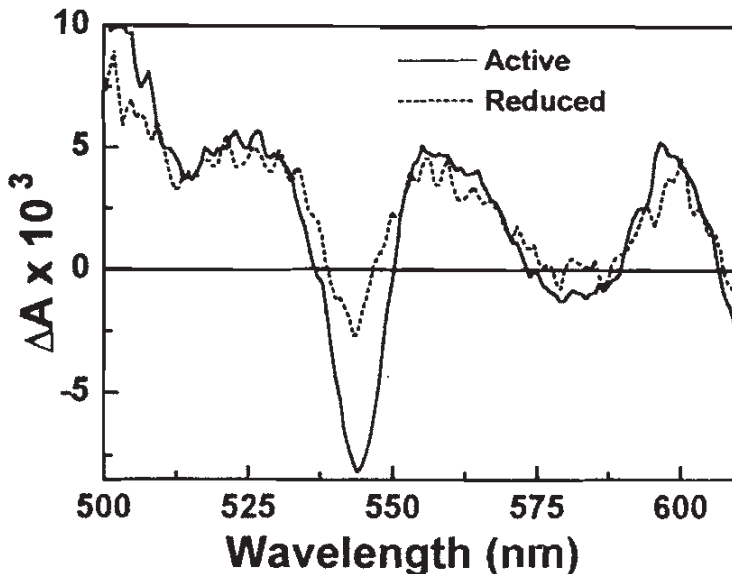


Figure 3. Transient absorption spectra of active and reduced (Pheo⁻ phototrapped) PSII RCs 500 ps after a 683-nm flash. On average, about half the active Pheo remained in a pre-reduced state over the 10 min it took to obtain the data.

(Fig. 1 inset) value at 500 ps reflects the amount of Pheo reduced by the pump flash. Independent EPR confirmation that our phototrapping technique forms Pheo⁻ was obtained. Figure 4 shows relative changes in amplitude of the shelf and the three kinetic components after phototrapping Pheo⁻. Although the data should be considered preliminary at this point, it appears that both the intermediate and slow components lose amplitude when Pheo is pre-reduced. If the samples are allowed to re-oxidize (indicated by a more negative shelf at 500 ps after the pump flash), the amplitudes of both intermediate and slow components increase. This experiment could be complicated by loss of function of some RCs in the sample since experiments (not shown) indicate that the amplitudes of all three kinetic components as well as their lifetimes decrease in “dead” centers.

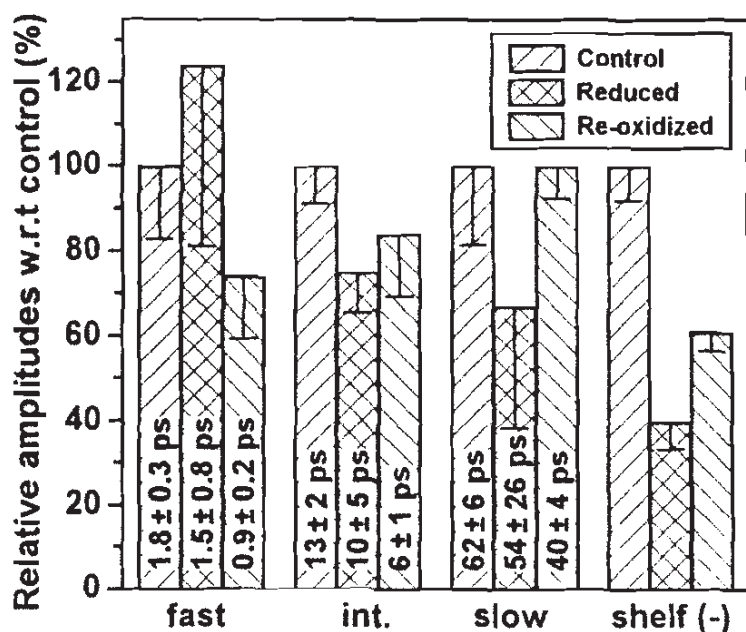


Figure 4. Changes in amplitude of the three kinetic components when Pheo is pre-reduced prior to a pump flash. Control means active samples. Re-oxidized samples were measured ca. 1 hr after phototrapping Pheo^- . The numbers on the columns are τ s. Half error bars are also shown.

In contrast to our previous report (2), we observe that pre-reduction of Pheo under our current conditions decreases somewhat the amplitude of the intermediate and slow components monitored at 544 nm, but not the fast (3-ps) component. However, since previous studies do not as yet eliminate the possibility that the fast component is associated with charge separation (2,6,7), we suggest that charge separation in isolated PSII RCs is heterogeneous and that the different transient absorption kinetic components monitored at 544 nm may all be convoluted functions of energy transfer and charge separation.

5. Acknowledgements

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