PICOSECOND SPECTROSCOPY OF PHOTOSYSTEM I REACTION CENTERS

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1. Introduction

When iron–sulfur centers A and B [1] (or P430 [2]), the stable primary electron acceptors of photosystem I reaction centers, are in the reduced state, photochemical electron transfer occurs between P700 and the earlier acceptors A_1 and A_2 [3]. Acceptor A_2 has been identified by EPR and optic spectroscopies as an iron–sulfur center [4]. Reduced A_2 has an EPR signal with g-values at 1.78, 1.88 and 2.08 [5,6], and it recombines with $P700^+$ with a halftime of 130 ms at 5 K [4].

The absorbance change at 694 nm (ΔA_{694}) measured 50 ps after excitation with a 694 nm excitation pulse is ~2-times that ascribable solely to P700 photooxidation; it decays in $\sim$ 200 ps to the level of $P700^+$ alone [7]. When iron–sulfur centers A and B are reduced, the ΔA has the same initial amplitude, but it decays in 2 phases with lifetimes of 10 ns and 3 μs [7]. The optic and EPR spectra of the 3 μs component are consistent with the assumption that photochemical charge separation occurs between P700 and a chl *a* dimer (electron acceptor A_1) [4,7]. This electron transfer apparently occurs in < 60 ps, followed by re-oxidation of the chl *a* dimer by P430 in $\sim$ 200 ps.

This work shows that the spectrum of the 200 ps decay component of the ΔA is consistent with that produced by the formation of an anion radical of a chl *a* dimer whose absorption-band maximum is at 695 nm.

2. Materials and methods

Triton-fractionated photosystem I subchloroplast fragments (TSF-I), prepared according to [8], had 1 P700/26 total chl molecules. The measurements were carried out under aerobic conditions in cuvettes with 1 or 2 mm pathlength. The particles were suspended in 0.1 M glycine buffer (pH 8) containing 1 mM ascorbate and 25 μM dichlorophenolindophenol (DCIP).

The setup for picosecond spectroscopy [9] consists of a passively mode-locked Nd-YAG laser, a single-pulse isolation system, and two Nd-YAG amplifiers. The single 30 ps pulse at 1060 nm (energy 30 mJ) was split into two beams: one was used to generate a ps continuum in D_2O and used as the measuring beam; the other (after frequency doubling) was passed through C_2D_5OD or liquid nitrogen to produce the stimulated Stokes Raman emission at 689 or 708 nm, which was utilized for selective excitation of the photosystem I reaction centers.

3. Results

Figure 1(a) shows the kinetics of ΔA_{694} induced by a 708 nm pulse in TSF-1 particles. The absorbance decrease occurs in < 30 ps, and decays from the maximum level in 2 phases with lifetimes of $\sim$ 45 ps and $\sim$ 210 ps to the level characteristic of P700 photooxidation alone. Decay kinetics with $t_{1/2} \sim$ 210 ps was also observed for ΔA_{484} (fig.1B) and 800 nm (not shown).

When P700 is pre-oxidized by continuous illumination, the 210 ps component is greatly diminished (fig.1A (b)) but the 45 ps component is unaltered. Heat-treated TSF-1 particles [10] produce an

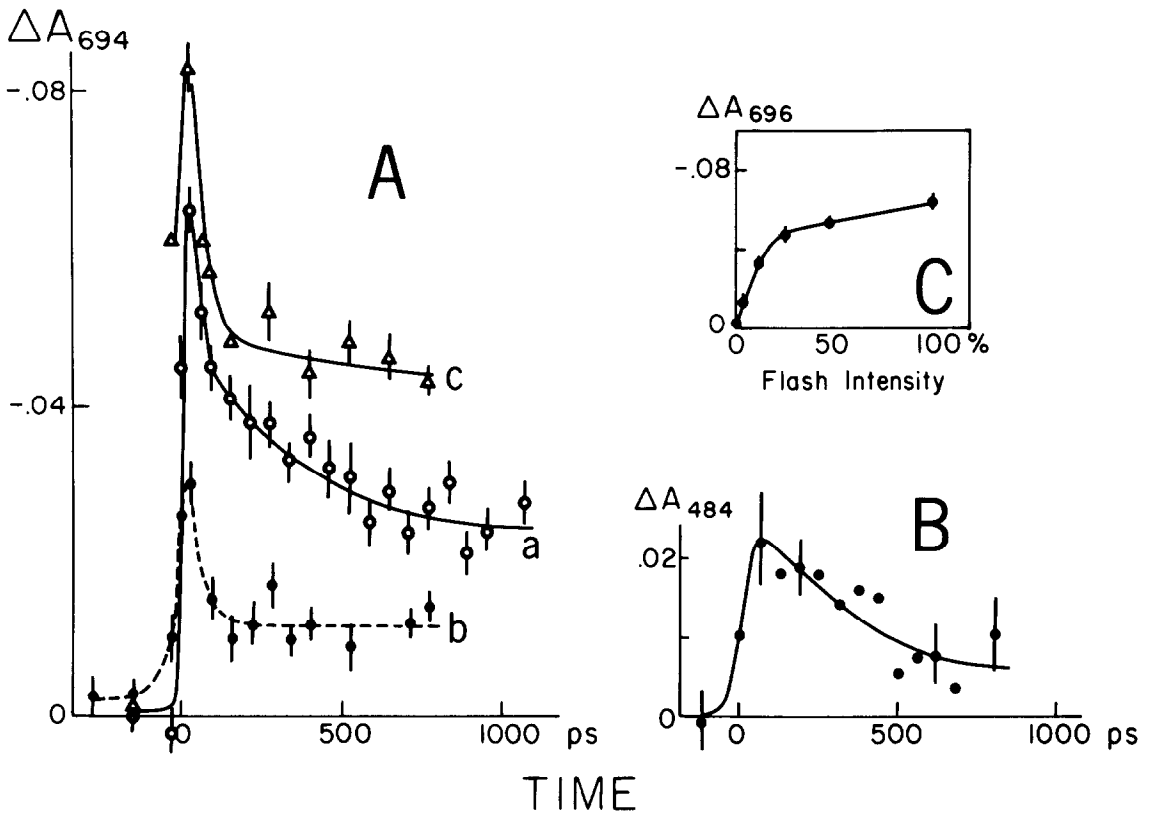

Fig.1. Kinetics of ΔA_{694} (A) and ΔA_{484} (B) in TSF-I particles. A (a), sample contained 1 mM ascorbate, 25 μM DCIP and 400 μg/ml chl (same sample used for ΔA_{484} except 800 μg/ml chl); A (b), sample contained 0.1 mM methyl viologen and 400 μg/ml chl; its P700 was pre-oxidized by continuous illumination; A (c), heat-treated TSF-1 particles [10], all other conditions same as for curve (a). The 708 nm excitation pulse was for 30 ps at 5×10^{15} quanta/cm^2. The inset (C) shows the flash-intensity dependence for ΔA_{694} measured 800 ps after excitation with the 708 nm pulse (maximum intensity (≡100%) = 10^{16} quanta/cm^2); sample was the same as used in ΔA_{484} measurements. All measurements were performed in a cuvette with 1 mm pathlength.

enhanced amplitude of the 45 ps component; the remaining amplitude is nearly the same as the 210 ps component in curve (a) and presumably represents the same process, but its lifetime is considerably lengthened (fig.1A (c)).

The flash-intensity dependence measured for ΔA_{694} (fig.1C) 1 ns after excitation with a 708 nm pulse indicates that the quantum yield of P700 photooxidation is ∿ 0.9 for low-intensity excitation light*.

Figures 2A,B show the difference spectra measured at 150 and 800 ps after excitation. The dashed spectrum in fig.2A,B is that for P700 oxidation by continuous illumination (i.e., [$P700^+$–P700]), which coincides with the data points measured at 800 ps, while the 150 ps spectrum is greater in amplitude. The difference between the spectra at 150 and 800 ps, shown in the inset of fig.2C, coincides well with that for the formation of chl *a* anion radical in solution [12] (shown by the dashed curve), except for a 30 nm shift toward the red. This shift may be accounted for by assuming that the chl *a* acceptor in photosystem I is dimeric [4,7], with an absorption-band maximum at 695 nm.

The 45 ps component absorbs mostly below 690 nm (not included in fig.2) and appears unaffected

* At 708 nm, the absorbance of P700 in the sample was 0.22. The quantum yield was calculated using 64 $mM^{-1} \cdot cm^{-1}$ as the mM extinction coefficient for P700 [11]

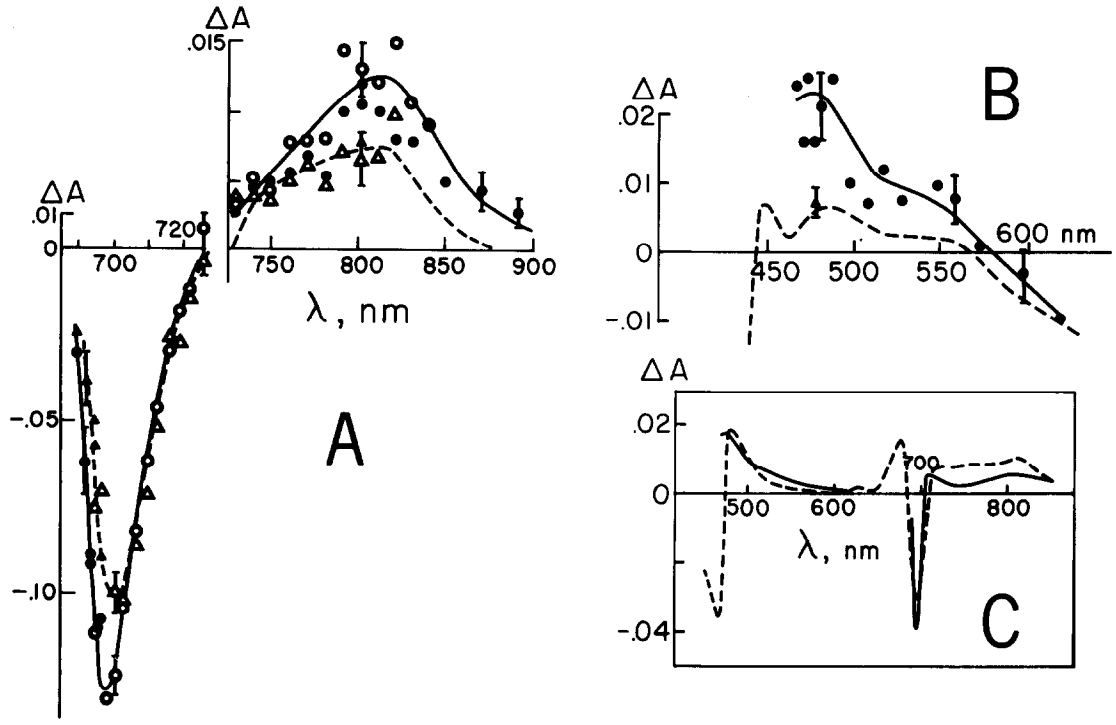

Fig.2. The spectra of ΔA in the red (A) and shorter-wavelength (B) regions measured 150 ps (○ and ●) and 800 ps (△ and ▲) after excitation with 708 nm (● and ▲) or 689 nm (○ and △) ps pulses in TSF-1 particles in the presence of 1 mM ascorbate and 25 μM DCIP. All measured values are normalized to 800 μg/ml chl and 1 mm pathlength. The dashed curve shows the difference spectrum of P700 measured in the same sample by continuous illumination. The inset (C) shows the difference between the 150 ps and the 800 ps spectra; the dashed curve is the difference spectrum (taken from [12]) for the formation of chl *a* anion radical, shifted toward the red by ~30 nm to coincide with the measured difference spectrum.

by prior oxidation of P700 (fig.1A (b)). This suggests that the 45 ps component is probably related to the excitation of antenna chl molecules. Fenton et al. [13] have also observed a ΔA with $t_{1/2} \sim 40$ ps in photosystem I particles which is insensitive to P700 photooxidation except the point at 730 nm. The sensitivity of the 40 ps component at 730 nm to P700 photooxidation may represent a band shift of antenna chl molecules, caused by the field associated with $P700^+$.

4. Discussion

The dependence of the 200 ps ΔA component on the redox state of P700 and P430 (also see [7]) shows it originates from the photochemical activity of photosystem I reaction centers. The spectrum of this component is consistent with the formation of an anion radical of the chl *a* (695) dimer. Presumably, light absorbed by photosystem I induces a transition in the excited assembly consisting of P700 and $(\text{chl } a)_2$ (695), which then relaxes to the state $[P700^+ \cdot (\text{chl } a)_2^-]$ in < 40 ps. The latter then transfers an electron to the iron–sulfur centers during $\sim$ 200 ps in a dark reaction [7]. In pre-reduced photosystem I particles, the reoxidation of $(\text{chl } a)_2^-$ takes considerably longer time. In this case, the reoxidation of $(\text{chl } a)_2^-$ probably follows the recombination route of $P700^+$ in 10 ns and 3 μs [7]. The extinction coefficient of the $(\text{chl } a)_2$ (695) acceptor was estimated to be 46 $nm^{-1} \cdot cm^{-1}$ from the ΔA extrapolated to $t = 0$, using 64 $mM^{-1} \cdot cm^{-1}$ as the mM extinction coefficient for P700 [11].

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