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The rate of formation of $P700^+ — A_0^-$ in photosystem I particles from spinach as measured by picosecond transient absorption spectroscopy

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Abstract. Photosystem I particles containing 30–40 chlorophyll *a* molecules per primary electron donor P700 were subjected to 1.5 ps low density laser flashes at 610 nm resulting in excitation of the antenna chlorophyll *a* molecules followed by energy transfer to P700 and subsequent oxidation of P700. Absorbance changes were monitored as a function of time with 1.5 ps time resolution. P700 bleaching (decrease in absorbance) occurred within the time resolution of the experiment. This is attributed to the formation of ^{1}P700.* This observation was confirmed by monitoring the rise of a broad absorption band near 810 nm due to chlorophyll *a* excited singlet state formation. The appearance of the initial bleach at 700 nm was followed by a strong bleaching at 690 nm. The time constant for the appearance of the 690 nm bleach is 13.7 $\pm$ 0.8 ps. In the near-infrared region of the spectrum, the 810 nm band (which formed upon the excitation of the photosystem I particles) diminished to about 60% of its original intensity with the same 13.7 ps time constant as the formation of the 690 nm band. The spectral changes are interpreted as due to the formation of the charge separated state $P700^+ — A_0^-$, where A_0 is the primary electron acceptor chlorophyll *a* molecule.

Abbreviations: A_0—primary electron acceptor of photosystem I, a chlorophyll *a* molecule; Chl—chlorophyll *a*; F_d—ferredoxin, a stable electron acceptor of photosystem I; P700—primary electron donor of photosystem I; PSI—photosystem I.

Introduction

Solubilization and subsequent specific fractionation of chloroplast membranes from higher plants and algae leads to the isolation of Photosystem I (PS-I) particles which are highly enriched in P700, the reaction center of PSI (see e.g. Kaplan and Arntzen, 1982). In the absence of exogeneous redox mediators an electron is transferred from P700, the primary electron donor, to the relatively

stable ferredoxin acceptor F_d (see e.g. Parson and Ke, 1982). The participation of a number of electron carriers prior to the reduction of F_d has been the subject of many investigations (for a recent review, see Rutherford and Heathcote, 1985). Optical measurements of transient absorption changes due to the formation of $P700^+$ and its associated reduced intermediate acceptors is complicated by the large number of antenna chlorophyll *a* (Chl) molecules that accompany the most stable P700 preparations. These particles typically contain at least 40 Chl molecules per P700.

Picosecond transient optical spectroscopy of P700 particles has with only one exception (Fenton et al., 1979) been confined to measurements using 35 ps laser pulses (Nuijs et al., 1986; and Shuvalov et al., 1986). It is now well known that the primary electron transfer events in bacterial reaction centers take place in less than 5 ps (see review by Holton and Kirmaier, 1987). Thus, it is important to examine the transient optical absorption changes that occur in P700 particles with short laser pulses. In addition, it is also important to limit the number of photons applied to the sample in order to avoid complications arising from the build-up of large numbers of Chl excited states in the antenna molecules associated with P700. The experiments presented here examine the nature of transient optical behavior of P700 with 1.5 ps time resolution and low photon fluxes.

Materials and methods

PS-I particles containing 30–40 Chl molecules per P700 were prepared as reported previously (Fenton et al., 1979). Picosecond time-resolved transient absorption measurements were obtained with the apparatus depicted in Fig. 1 as follows: P700 particles were placed in a 1 mm pathlength cell. The absorbance of the sample was 1.2 at 680 nm. Two sets of measurements were performed. In one set the PS I particles were suspended in a buffer containing the exogenous reductant reduced dichlorophenolindophenol (DPIP/ascorbate) to re-reduce $P700^+$ prior to the next laser pulse, while in the other set no reductants were added. Subtraction of the transient absorption changes obtained from these two samples eliminates changes due entirely to the formation of excited states of the residual antenna Chl molecules.

A 2-mm diameter spot on the sample cell was illuminated with the pump and probe beams of the transient absorption apparatus. The 514 nm output of a mode-locked Ar^+ laser operating at an 82 MHz repetition rate was used to synchronously pump a rhodamine-6G dye laser, which resulted in 610 nm, 1.5 ps, 1 nJ pulses from the dye laser. These pulses were amplified to 2.5 mJ using a 4-stage rhodamine-640 dye amplifier pumped by a frequency-doubled Nd-YAG laser operating at 10 Hz. The resulting 1.5 ps amplified laser pulse was split with a dichroic beam splitter. A 610 nm, 1.5 ps, 100 μJ pulse was used to excite the samples. The remaining 610 nm, 1.5 ps, 2.4 mJ pulse was used to generate a 1.5 ps white light continuum probe pulse. Pulse lengths were determined by autocorrelation techniques. The total instrument respopnse function was 1.5 ps. Typically, 256 laser shots were averaged to obtain the data presented here. Transient absorbance measurements were made with a double beam spectrometer which employed optical multichannel detection. Time delays between pump and probe pulses were accomplished with an optical delay line. Time constants for kinetic

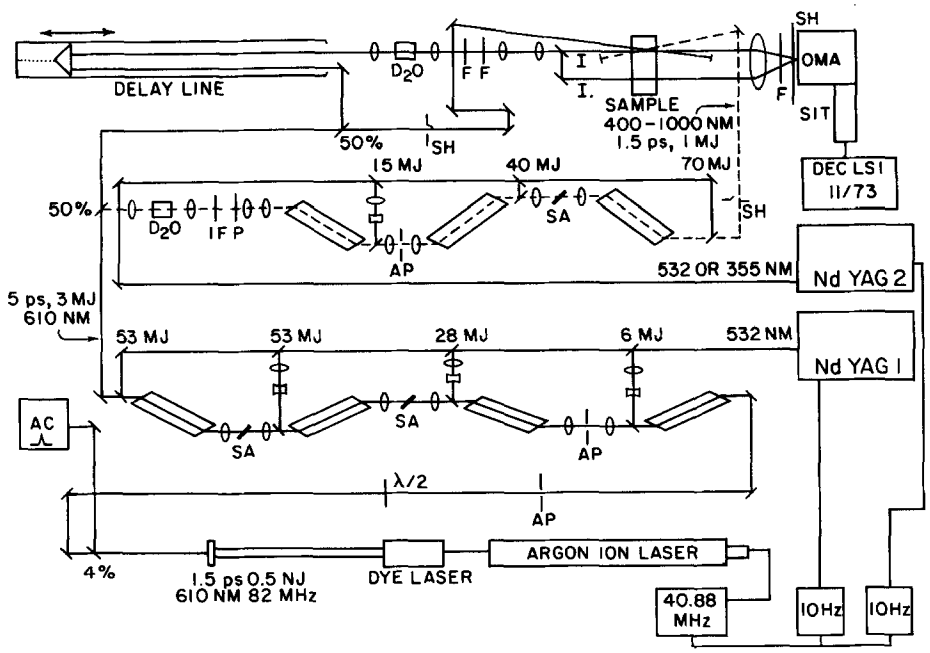

Fig. 1. Picosecond absorption spectrometer used in the present study (see text). AP, aperture; DEC LSI, Digital Equipment Corp. 11/73 computer; F, filters; IF, interference filter; Nd-YAG, Quanta Ray DCR-2 Nd-YAG$_2$ Molectron MY-34; OMA, optical multichannel analyzer; P, polarizer; SH, shutter; SIT, intensified video camera. The time resolution of the apparatus was 1.5 ps for the measurements presented in this paper.

data were determined by iterative reconvolution using the Grinvald-Steinberg method (Grinvald and Steinberg, 1976).

Results

Figure 2 displays a series of transient absorption spectra obtained as a function of time following a 1.5 ps laser pulse at 610 nm. Spectral changes due purely to antenna processes have been subtracted out of these spectra as detailed in the Materials and Methods section. A bleach (decrease in absorbance) at 700 nm appears initially and can be ascribed to any process that depletes ground state P700, including both ^{1}P700* and P700$^+$ formation. This bleach persists and is followed later in time by the formation of an additional bleach at 690 nm. A prominent shoulder also appears at 675 nm.

The time dependence of the bleaches at 700 nm and at 690 nm are shown in Figs 3 and 4, respectively. From the data in Fig. 3 it is clear that P700 bleaches as fast as the 1.5 ps instrument response time. This bleach persists over the 50 ps time window measured. On the other hand, the bleach at 690 nm depicted in Fig. 4 appears more slowly and can be fitted with a single exponential decay constant of 13.7 $\pm$ 0.9 ps.

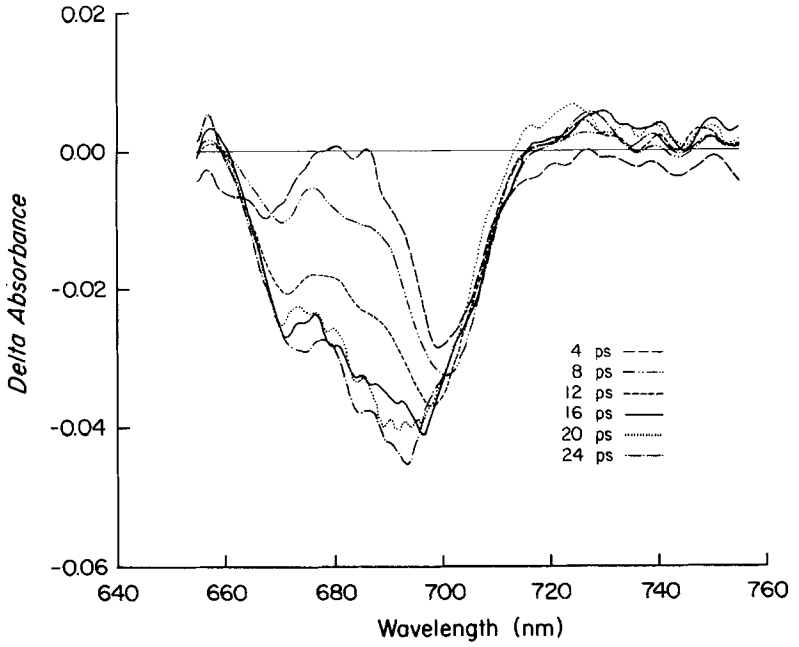

Fig. 2. Wavelength and time dependences of transient absorbance changes of PS-I particles (with antenna changes subtracted) following excitation with 1.5 ps, 610 nm laser pulse.

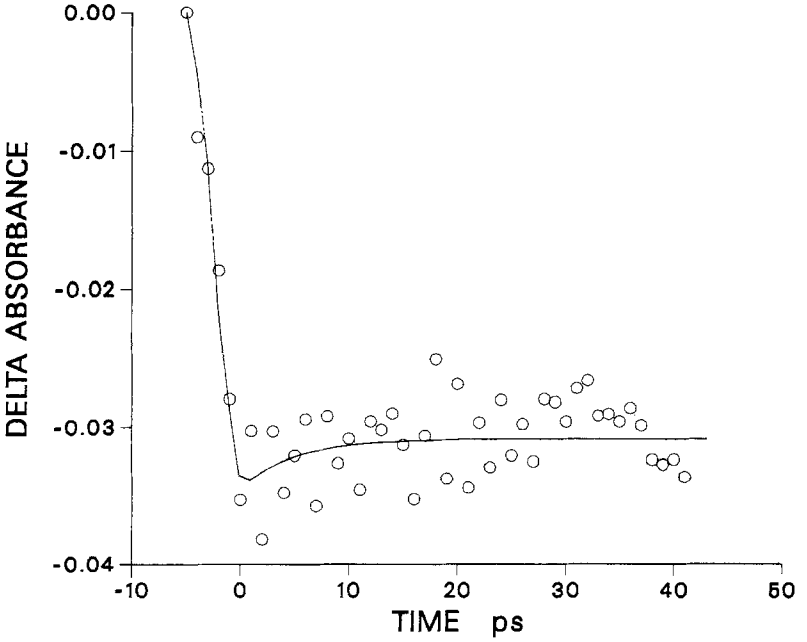

Fig. 3. Time dependence of transient absorbance change at 700 nm for PS-I particles (with antenna changes subtracted) following a 1.5 ps, 610 nm laser pulse.

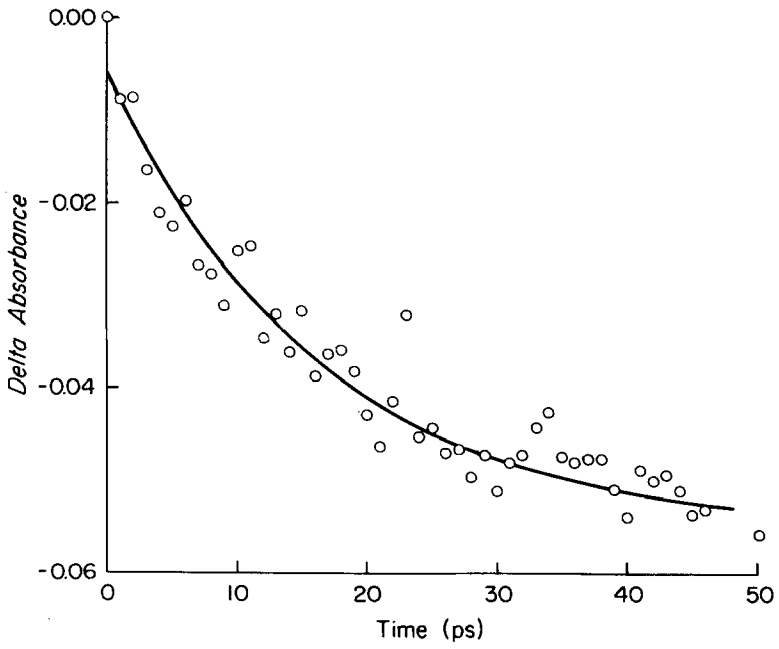

Fig. 4. Time dependence of absorbance change at 690 nm of PS-I particles (with antenna changes subtracted) following a 1.5 ps, 610 nm laser pulse.

The corresponding absorption changes in the near-infrared spectral region were also measured. A broad absorption band centered at 810 nm appears immediately upon excitation of P700, Fig. 5. The magnitude of this band changes as a function of time as depicted in Fig. 6. The decay of the 810 nm band, displayed in Fig. 6, to a constant absorbance value occurs with the same 13.7 ps time constant as did the appearance of the 690 nm bleach.

Discussion

Both the appearance of the bleach at 700 nm and the positive absorption change at 810 nm occur as fast as the 1.5 ps instrument response. Bleaching of the Q_y band of chlorophylls occurs whenever ground state population is lost irrespective of whether excited or ionic states are formed. In addition, both the lowest excited singlet state of chlorophylls and their cation radicals possess significant absorption in the 800 nm region. The picosecond transient absorption spectrum of Chl *a* shows that the lowest excited singlet state of Chl *a* possesses an absorption band in the 800 nm region (see e.g. Huppert et al., 1976). The corresponding transient absorption spectrum of the lowest excited singlet state of methyl pyrochlorophyllide *a* is shown in Fig. 7 to illustrate this point. Thus,

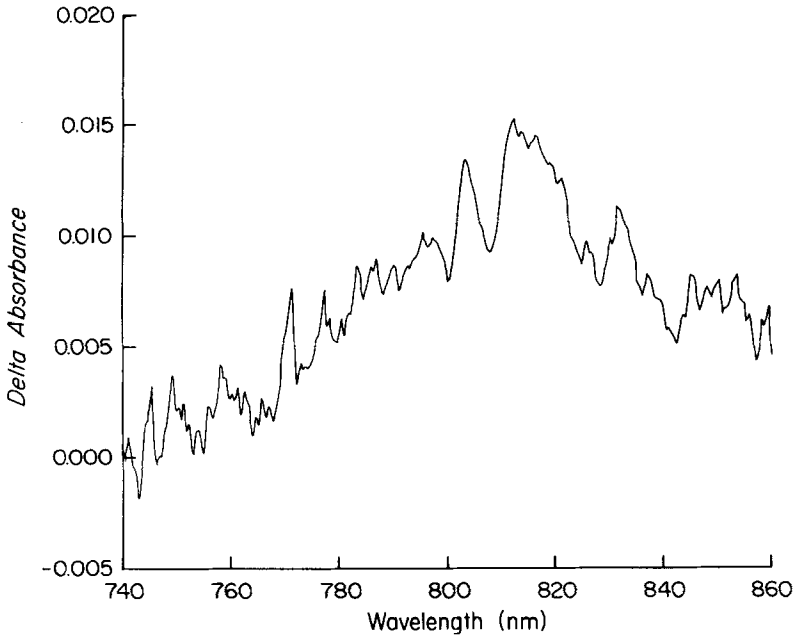

Fig. 5. Wavelength dependence of transient absorbance change of PS-I particles (with antenna changes subtracted) at 0 ps following a 1.5 ps, 610 nm laser pulse.

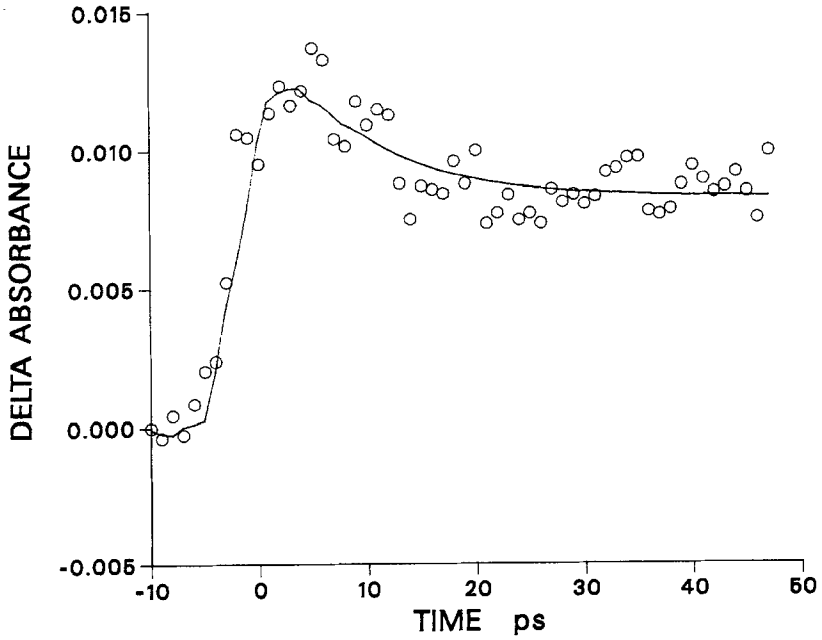

Fig. 6. Time dependence of absorbance change at 810 nm for PS-I particles (with antenna changes subtracted) following a 1.5 ps, 610 nm laser pulse.

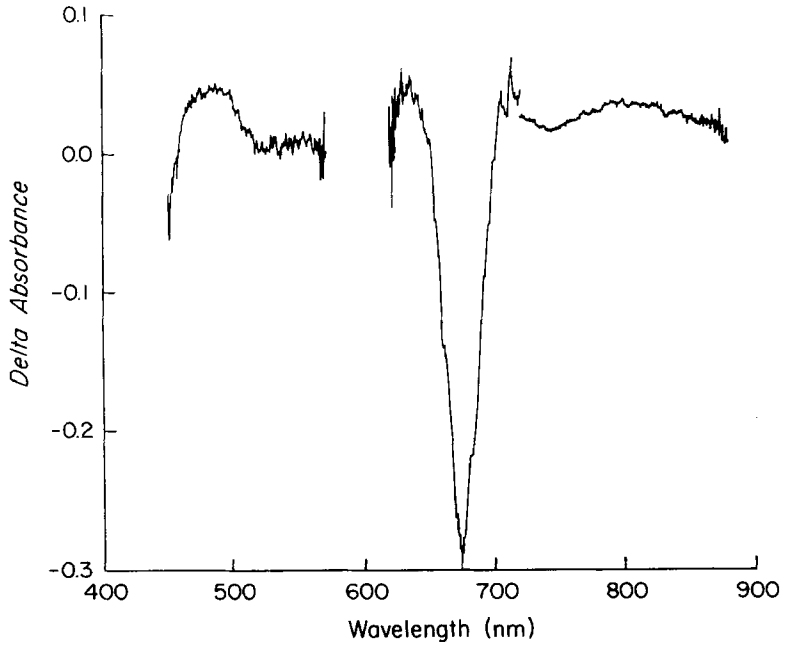

Fig. 7. Transient difference absorption spectrum of methyl pyrochlorophyllide *a* in butyronitrile at 0 ps following a 1.5 ps, 610 nm laser pulse.

we attribute the immediate ($\leqslant 1.5$ ps) bleach of P700 and formation of the 810 nm band to the excitation of P700 to ^{1}P700.*

Since the absorbance of antenna Chl *a* in these PS-I particles is $\geqslant 30$ times that of P700 at the 610 nm excitation wavelength; it is likely that the observed formation of ^{1}P700* is due to energy transfer from the antenna rather than direct excitation of P700. Therefore, the $\leqslant 1.5$ ps formation time of ^{1}P700* that we measure can be interpreted as an upper limit for the time constant for energy transfer to P700 from the antenna Chl *a* molecules closely associated with P700.

We attribute the appearance of a bleach at 690 nm concomitant with a small decrease in absorbance at 810 nm to the formation of $P700^+ — A_0^-$. The bleach at 690 nm which appears with a 13.7 ps time constant is similar to that observed by Nuijs et al. (1986) and by Shuvalov et al. (1986). However, the measurements performed by these workers had a time resolution of 35 ps and thus the kinetics of the appearance of this band were not observed. The assignment of A_0 to a Chl-like species can be made by comparing the transient spectra obtained here with the optical spectra of the radical anions of chlorophyll *a* and pheophytin *a* (Fujita et al., 1978).

The fact that the reduction of A_0 is relatively slow, i.e., about 14 ps, leads us to speculate that there may be other as yet undetected electron acceptors which are reduced by ^{1}P700* prior to the formation of A_0^-. Recently, sub-picosecond transient absorption measurements were carried out on the reaction center proteins of *Rhodobacter sphaeroides* R-26 (Woodbury et al., 1985, Martin et al.,

1986) and *Rhodopseudomonas viridis* (Breton et al., 1986, Wasielewski and Tiede, 1986). These studies showed that the bacteriopheophytin anion is the first observable reduced intermediate in these proteins. The reduction of bacteriopheophytin occurs in 2.8 ps in native reaction centers from both organisms, while in reaction centers in which quinone Q_A is pre-reduced, this reduction occurs in 5.4 ps for *Rb. sphaeroides* R-26 and in 6.0 ps for *Rps. viridis*. Since the PSI particles used in the experiments presented here have been treated with an exogenous reductant, the bacterial results suggest it is possible that the observed 13.7 ps formation time for A_0^- may be somewhat longer than the corresponding time in untreated particles. More careful spectroscopic work will be needed to investigate these possibilities.

In conclusion, the current ***working hypothesis*** for the photochemistry of Photosystem I is:

1) Chl *a* (antenna) + h2 $\xrightarrow{t_{\frac{1}{2}} \sim 1\,\text{fs}}$ Chl *a** (antenna)
2) Chl *a** (antenna) + ^{1}P700 $\xrightarrow{t_{\frac{1}{2}} \leq 1.5\,\text{ps}}$ Chl *a* (antenna) + ^{1}P700* (this paper)
3) ^{1}P700* + A_0 (Chl *a*) $\xrightarrow{t_{\frac{1}{2}} \sim 14\,\text{ps}}$ ^{1}P700$^+$ + A_0^- (Chl *a*) (this paper; also see Nuijs et al. 1986, Shuvalov et al. 1986)
4) A_0^- (Chl *a*) + A_1 (Quinone) $\xrightarrow{t_{\frac{1}{2}} \sim 40\,\text{ps}}$ A_0 (Chl *a*) + A_1^- (Quinone) (see e.g., Fenton et al. 1979 for a 40 ps component, and Petersen et al. 1987 for A_1 being a quinone)

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