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Picosecond absorbance changes upon selective excitation of the primary electron donor P-700 in Photosystem I

V.A. Shuvalov *, A.M. Nuijs **, H.J. van Gorkom, H.W.J. Smit and L.N.M. Duysens

Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden (The Netherlands)

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Selective picosecond excitation of P^+-700, at 710 nm, was used to study the primary processes in reaction centers in relatively intact Photosystem I particles. The transient formation of the state P^+-700C^--693 was observed. The absorption decrease at about 693 nm was attributed to the reduction of a chlorophyll *a* molecule, C-693. C^--693 had a lifetime of 32 ± 5 ps. When the electron transfer was blocked by the reduction of the secondary acceptors the state P^+-700C^--693 decayed in 20–30 ns by recombination, with a triplet yield of 30%.

Introduction

Application of picosecond spectroscopy to small Photosystem I particles obtained by a rather drastic treatment has previously shown that the excitation transfer from antenna chlorophyll to the reaction center occurs in 30–40 ps and leads to a charge separation which produces P^+-700 (P^+) and chlorophyll a^- (C^-) [1,2]. When the iron–sulfur centers which normally act as secondary electron acceptors in PS I were in the reduced state, the lifetime of P^+C^- was estimated to be 10–25 ns [1,3]. When the iron–sulfur centers were oxidized in these small particles (25 chlorophylls/P-700) the electron transfer from C^- to those centers occurred within about 200 ps [2], but in a more intact Photosystem I preparation containing about 100 chlorophylls/P-700 the state P^+-700C^--693 could not be observed at about 50 ps after excitation [3]. At shorter time large absorbance changes due to the excited antenna chlorophyll would have prevented its detection.

Therefore in this work the selective ps excitation of the primary donor of the Photosystem I particles (100 chlorophylls/P-700) at 710 nm was used to decrease the antenna excitation considerably. Under such conditions the state P^+-700C^--693 could be detected also in these particles and was found to have a lifetime of 32 ± 5 ps at moderate potential and of 20–30 ns under reducing conditions.

Materials and Methods

Triton-fractionated Photosystem I subchloroplast fragments, prepared according to Ref. 3, with about 100 chlorophylls/P-700 were used in picosecond experiments. The particles were suspended in 50 mM Tris-HCl buffer (pH = 8.0) in

* Permanent address: Institute of Soil Science and Photosynthesis, USSR Academy of Sciences, Pushchino, Moscow Region, U.S.S.R.
** To whom correspondence should be addressed.

Abbreviations: C-693, chlorophyll 693; P-700, primary electron donor chlorophyll 700; PMS, *N*-methylphenazonium methosulfate; PS, Photosystem.

the presence of ascorbate (1 mM) and PMS (10 μM) for experiments with oxidized iron–sulfur centers, and were suspended in 0.1 M glycine buffer (pH = 9.5) containing PMS (10 μM) and dithionite (10 mM) upon background illumination for measurements with reduced iron–sulfur centers.

For the picosecond measurements a passively mode-locked Nd-YAG laser with a single-pulse isolation system and two Nd-YAG amplifiers was used. The single 35-ps pulse at 1064 nm (see Ref. 4) was split into two beams: one was frequency-doubled and was passed through liquid nitrogen to produce the stimulated second Stokes–Raman emission at 710 nm which was used for selective excitation of P-700. The second beam was focussed in a mixture of H_2O and ^2H_2O to generate a ps continuum which was split into a measuring beam passing through the sample and a reference beam by-passing it. An optical multichannel analyzer (OMA-2, EG&G) was used to measure simultaneously the two spectra obtained by passing the measuring and reference pulse at different height through the same polychromator. The results are presented in the form of difference (light-minus-dark) absorption spectra, measured at different delay times with respect to the excitation at 710 nm. In some experiments the sample was preilluminated with a ps pulse at 532 nm at 2 ns before the excitation at 710 nm, by deflecting part of the frequency-doubled beam towards the sample via a shortcut. Picosecond measurements were done at room temperature in 1- or 2-mm cuvettes.

Microsecond absorbance changes were measured in a single-beam spectrophotometer with a time resolution of 0.5 μs. Excitation flashes of 15 ns half-width, 590 nm, 0.4 mJ/cm^2 were provided at a repetition rate of 2.5 Hz by a Rhodamine B dye laser, pumped by a Q-switched, frequency-doubled Nd-YAG laser. The measuring light obtained from a 1000 W xenon lamp passed through a monochromator, a chopper, the sample (optical pathlength 2.8 mm) and a second monochromator. The transmitted light was measured with a photomultiplier (EMI 9558 C), protected from stray actinic light by a Schott RG 630 filter, and the signal was fed into a transient recorder (Biomation model 8100). Fluorescence artefacts were recorded separately and subtracted.

Results

Figs. 1A and B show the absorption difference spectra in Photosystem I particles at moderate potential measured at 5 ns and 880 ps after the excitation at 710 nm. Both spectra mainly represent the (P^+-700–P-700) spectrum with a maximum bleaching at 700 nm and a small bleaching near 683 nm. The absorbance changes at 690 nm were close to zero. The contribution by the chlorophyll triplet state is very small, in contrast to the measurements using excitation at 532 nm [3]. Apparently, at 880 ps after the flash the electron is transferred to an acceptor, the reduction of which does not give any measurable absorbance changes in this spectral region, in agreement with earlier

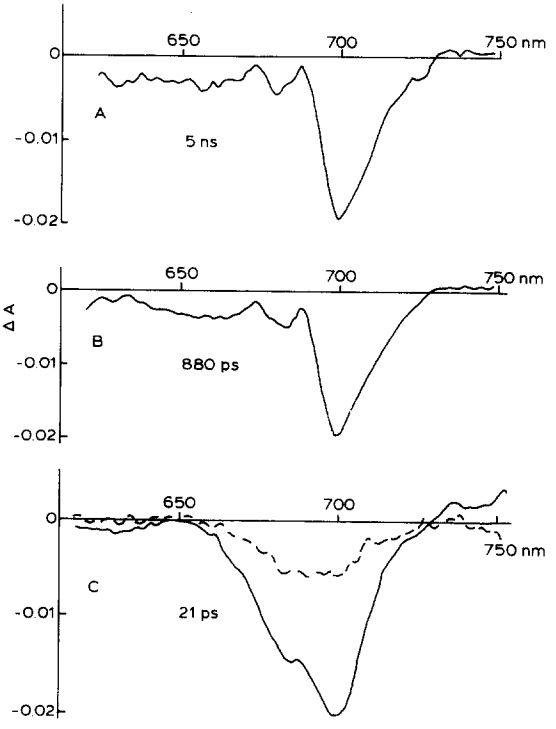

Fig. 1. Absorption difference spectra of Photosystem I subchloroplast fragments (A_{680} = 1.4) in the presence of ascorbate (1 mM) and PMS (10 μM). Absorbance changes were measured at 5 ns (A), 880 ps (B) and 21 ps (solid curve in C) after the center of the 35-ps excitation pulses at 710 nm. The dashed curve in (C) shows the spectrum measured as for the solid curve but with a 532 nm preflash (excitation energy density, about 0.5 mJ/cm^2) at −2 ns to oxidize the main part of P-700 before the excitation at 710 nm.

data [5], and no re-reduction of P^+-700 is observed in the time range of 1–5 ns.

The spectrum measured at 21 ps (Fig. 1C), when the bleaching at 700 nm approaches the maximum constant level, is characterized by an additional bleaching in the region 680–700 nm. This additional bleaching might be due either to the antenna chlorophyll excitation [3] or to the charge transfer in reaction centers. To discriminate between these possibilities the sample was excited by a 532 nm pulse at about 2 ns before the excitation at 710 nm. The preflash at 532 nm should induce the oxidation of most of P-700 and thereby suppress absorbance changes resulting from P-700 excitation by the 710 nm flash, whereas absorbance changes resulting from direct excitation of antenna chlorophylls should not be affected. The result of these measurements is shown in Fig. 1C by the dashed curve. The spectral shape is close to that obtained without preflash (solid curve). The amplitude of the bleaching at 700 nm is smaller by a factor of 3.5, indicating that the preflash caused the oxidation of about 70% of P-700. The amplitude at 680 nm is decreased by about 60%, indicating that only a small fraction ($\approx 10\%$) of it can be ascribed to direct excitation of antenna chlorophyll. At 690 nm, where the contribution of P-700 oxidation is negligible, more than 90% of the bleaching must be attributed either to the reduction of an early electron acceptor or to excited states produced by excitation of P-700 itself.

The decay kinetics of the bleaching at 690 nm are shown in Fig. 2. The deconvolution of the kinetics (dashed curve) using the 35-ps Gaussian pulse shape shows that a reasonable fit is obtained using a lifetime of 32 ± 5 ps.

If this lifetime is determined by electron transfer to a secondary acceptor, it should be increased by reducing that acceptor before the excitation. The reduction of the secondary acceptors in Photosystem I particles was carried out in the presence of dithionite and continuous illumination.

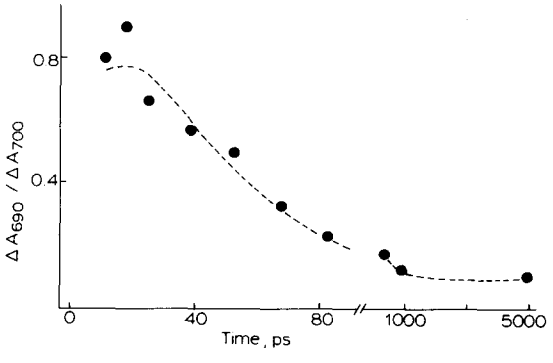

Fig. 2. Kinetics of the absorbance changes at 690 nm divided by those at 700 nm in Photosystem I particles excited at 710 nm. Conditions as for Fig. 1. The dashed curve shows the calculated kinetics using the 35-ps pulse shape and a 32-ps decay time.

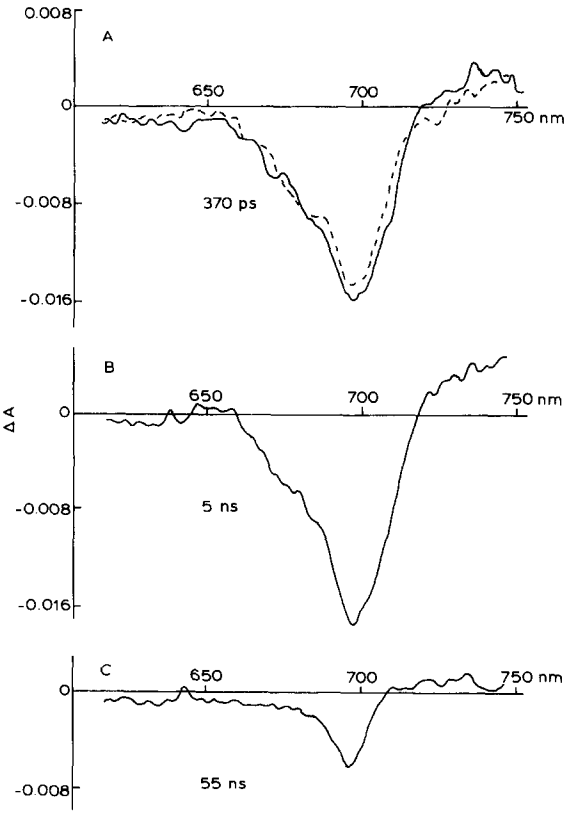

Fig. 3. Absorption difference spectra of Photosystem I particles ($A_{680} = 1.1$) in 0.1 M glycine buffer (pH = 9.5) containing PMS (10 μM) and dithionite (10 mM) in the presence of background illumination to reduce the iron–sulfur centers. Absorbance changes were measured at 370 ps (solid), 5 ns and 55 ns after the center of the excitation pulse at 710 nm. The dashed curve in Fig. 3A shows the difference between the spectra shown in Fig. 1C.

The picosecond measurements of absorption difference spectra under such conditions at 370 ps and 5 ns are shown in Fig. 3A and B. These spectra are very similar to each other and include the bleaching at 700 nm as well as that at 690 nm. The same spectrum was obtained previously using a 532 nm excitation pulse [3]. The spectrum measured at 55 ns (Fig. 3C) is different and closely resembles the spectra of the triplet state of P-700 measured in the microsecond time domain ($\tau = 3 \pm 0.2$ μs) at 293 K (Fig. 4) and at low temperature [5,12]. The amplitude indicates that at 55 ns about 30% of P-700 was in the triplet state.

The state present at earlier times is spectrally the same as that observed initially upon excitation of P-700 under non-reducing conditions (dashed line in Fig. 3A, obtained by taking the difference between the 21 ps spectra without and with preflash of Fig. 1C). This state, which normally generates a stable charge separation between P-700 and an undetected electron acceptor, such as an iron–sulfur center, presumably F_X [5], decays in 32 ± 5 ps (Figs. 1 and 2), thus appears to be stabilized about 1000-fold (Fig. 3B) by conditions

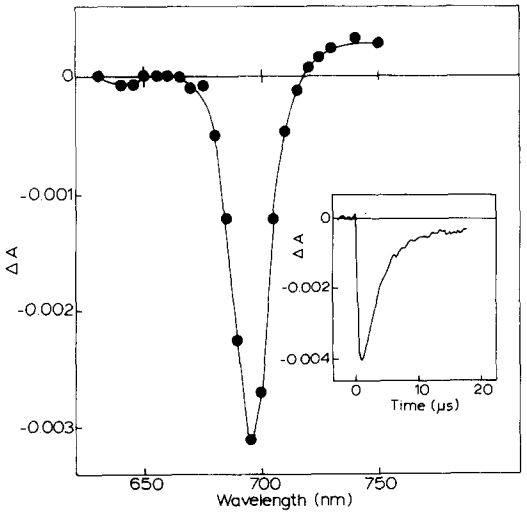

Fig. 4. Spectrum of flash-induced absorbance changes with a lifetime of 3 μs in Photosystem I particles ($A_{680} = 1.4$) in 250 mM glycine buffer, containing 15 mM dithionite in the presence of background illumination; to maintain anaerobic conditions, glucose, glucose oxidase and catalase were added. Actinic flashes were not saturating. Only the 3 μs component was plotted. Inset: kinetics of the absorbance changes at 695 nm.

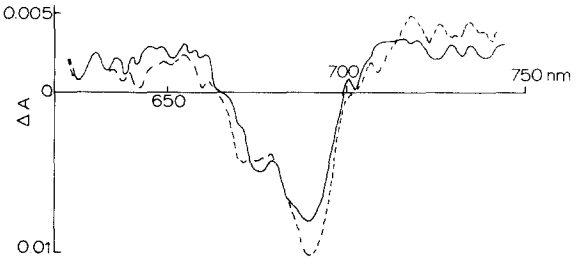

Fig. 5. Solid and dashed curves show the result of the subtraction of the spectrum of P^+-700–P-700 (Fig. 1A) from that shown in Fig. 3A for moderate (solid) and low (dashed) potentials, respectively, after normalization at 700 nm. This normalization at 700 nm is justified by the observation in Fig. 1 that the amplitude of the bleaching at 700 nm does not depend on the delay from 21 ps to 5 ns at moderate potentials, while the additional bleaching at 690 nm fully decays in that time.

which cause a reduction of the iron–sulfur centers normally acting as electron acceptors in PS I. Under these conditions the state is lost within 55 ns, leaving 30% of P-700 in the triplet state, P^T-700 (Fig. 3C). The simplest interpretation of these data is that the absorbance difference spectra of Fig. 3A represent the oxidation of P-700 plus the reduction of a primary electron acceptor; if the secondary acceptor is prereduced, the radical pair is partly converted to P^T-700.

Subtraction of the difference spectrum of P-700 oxidation (Fig. 1A) from the spectra of Fig. 3A should then yield the difference spectrum due to reduction of the primary electron acceptor in PS I. The result (Fig. 5) is a difference spectrum with a pronounced bleaching at 690 nm with a shoulder at 675 nm (the origin of which will be discussed later), flanked on both short- and long-wavelength sides by a smaller, rather flat absorbance increase.

Discussion

In agreement with the previous suggestions derived from the picosecond measurements at moderate potential [1,2] and under reducing conditions [3] the spectra presented in Fig. 5 (disregarding the shoulder at 675 nm, see below) are similar to the spectrum of chlorophyll radical anion formation measured in solutions [6] if red-shifted by about 25 nm. Therefore, the primary process of the charge separation in Photosystem I appears to

include the formation of P^+-700 and chlorophyll a^--693 (C^--693). The state P^+-700C^--693 has a lifetime of 32 ± 5 ps in the presence of oxidized iron–sulfur centers, in agreement with Ref. 3, but different from that of about 200 ps measured earlier for Photosystem I particles which had 25 chlorophylls/P-700 [1,2]. This probably reflects a decrease of the electron transfer rate from C^--693 to the secondary acceptor, from $1/32$ ps^{-1} to $1/200$ ps^{-1}, upon extensive fractionation of Photosystem I particles with Triton X-100. In reduced samples the lifetime of both P^+-700 and C^--693 was 20–30 ns, confirming the interpretation by Sétif et al. [7] of a 30–50 ns phase in the decay of the absorbance change at 815 nm. The spectra in Fig. 5 also include a small shoulder at 675 nm, which was not observed previously [3]. This may be due to some contribution of a triplet state of antenna chlorophyll, in spite of the 710 nm excitation and the subtraction of the (P^+-700–P-700) spectrum, or it may have been missed in Ref. 3.

The recombination of P^+-700C^--693 in the presence of dithionite leads to the formation ($\phi \approx$ 30%) of the triplet state of Photosystem I reaction centers. Optical and EPR spectra at low temperature of these states have been described earlier [5,8,11]. The spectrum of the triplet state (Fig. 4) is different from that of (P^+-700–P-700) and has the maximum bleaching at 696 nm with a symmetrical shape on both long- and short-wavelength sides. The long-wavelength isobestic point is shifted from 725 to 717 nm. If the triplet state is localized purely on P-700, these differences are perhaps due to electrochromic contributions to the more complicated spectrum of (P^+-700–P-700). The triplet state, on the other hand, may contain contributions by C^T-693, or $[P^+C^-]^T$.

Thus the primary charge transfer state in reaction centers of Photosystem I is the state P^+-700C^--693 which may be comparable to the state P^+B^- in bacterial reaction centers (Refs. 4, 9 and 10; see also Shuvalov, V.A., Vasmel, H., Amesz, J. and Duysens, L.N.M., unpublished results), where B is a monomeric bacteriochlorophyll *a* or *c* molecule. In more intact Photosystem I particles the state P^+-700C^--693 transfers an electron to a secondary electron acceptor in about 32 ps, while the state P^+B^- transfers an electron to bacteriopheophytin in about 1.5–3 ps in reaction centers of purple bacteria and in the green bacterium *Chloroflexus aurantiacus* [4,11]. The time constant of the reoxidation of $BChl^-c$ in the green sulfur bacterium *Prosthecochloris aestuarii* and in *Heliobacterium chlorum* is 500–600 ps [9,10].

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