KINETIC AND SPECTRAL PROPERTIES OF THE INTERMEDIARY ELECTRON ACCEPTOR A_1 IN PHOTOSYSTEM I

Subnanosecond spectroscopy

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1. Introduction

In recent studies of green-plant photosystem I, evidence has been found for the existence of two earlier electron acceptors, designated A_1 and A_2, functioning between the primary donor, P700, and the acceptor P430, found [1], provisionally represented by the sequence ([2,3] also cf. [4]):

P700–A_1–A_2–P430

When P430 (bound iron–sulfur protein) is chemically reduced beforehand, charge recombination is observed between $P700^+$ and A_1^- or between $P700^+$ and A_2^- following flash excitation, with lifetimes of 3 μs [2–5] and 250 μs [4] at 20°C, becoming 1.3 ms [2,3,5] and 130 ms [2,3] at 5 K, respectively. The spectra obtained for the two intermediary acceptors have led us to identify A_1 with a chlorophyll-*a* dimer and A_2 with an iron–sulfur protein [2,3]. A broad EPR signal, designated X [6,7], has been identified with the optical signal of A_2 on the basis of parallel kinetic behavior [2].

This note reports the ps kinetics $\Delta A_{694.3}$ in TSF-I (Triton-fractionated photosystem-I subchloroplast fragments) particles at room temperature, induced by single 50 ps pulses at 694.3 nm, absorbed mainly by P700 rather than antenna chlorophyll molecules. The initial absorbance change, which subsequently decays to the level of photo-oxidized P700 alone, is interpreted as formation of the charge pair $P700^+ \cdot Chl_2^-$ in <60 ps, followed by reoxidation of the chlorophyll-*a* dimer radical anion by a secondary electron acceptor in ~200 ps. When P430 is chemically reduced, the initial ΔA is the same, but the decay is slower and biphasic, and is interpreted as due to recombination between $P700^+$ and Chl_2^- in 10 ns and 3 μs.

2. Materials and methods

TSF-I, prepared as in [10], had 1 P700/26 total chlorophyll molecules. Redox mediators were as in [11].

For ps kinetic measurements at 694.3 nm a passively mode-locked ruby laser was used (fig.1). A single pulse at 694.3 nm was isolated from a ps pulse train by a Glan prism and a Pockels cell energized for 6–7 ns by a high-voltage pulse initiated by breakdown across the spark gap. 50% of the single pulse energy was used as exciting light and 1–2% as measuring light. One of the measuring beams was passed through an unexcited region of the sample to serve as a reference beam. The duration of the exciting pulse was 50–60 ps as measured by cryptocyanine bleaching (fig.1 bottom left).

For μs and ms measurements, a 300 ns dye-laser pulse at 710 nm (Nile Blue perchlorate) was employed

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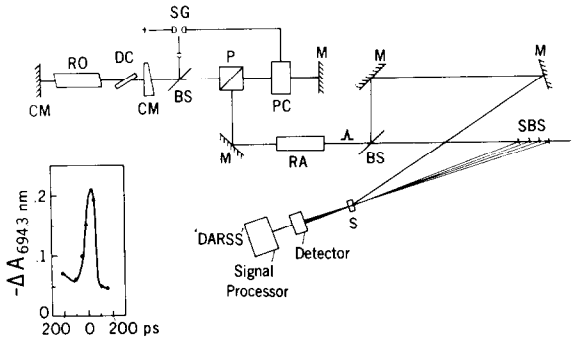

Fig.1. A picosecond spectrometer consisting of a passively mode-locked ruby laser, a single-pulse isolation system, an amplifier, an optical delay line, and a photodiode array and signal processing system manufactured by Tracor Northern Inc. (Middleton, WI). Components: CM-cavity mirrors; RO-ruby oscillator; DC-mode locking dye cell; BS-beam splitters; P-polarizing beam splitter; PC-Pockels cell; RA-ruby amplifier; M-mirrors; SBS-stacked beam splitters; S-sample cuvette (1 mm pathlength). Bottom left shows the kinetics of ΔA cryptocyanine in acetonitrile at 694.3 nm induced by a 694.3 nm ps excitation pulse.

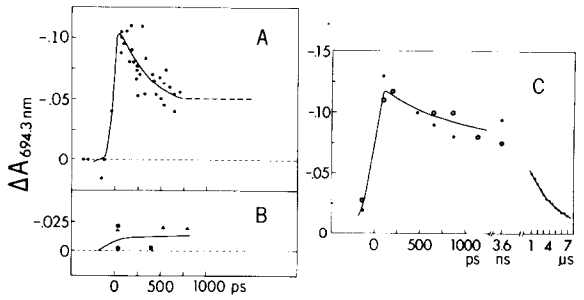

Fig.2. Kinetics of $\Delta A_{694.3}$ in TSF-I particles induced by single 694.3 nm pulses (50–60 ps duration and an energy density of 1 mJ/cm^2) at 20°C. Each point of the kinetic plot was an average of 10–20 measurements. (A) Aerobic reaction mixture in 0.1 M Tricine buffer (pH 8.0) containing 1 mM ascorbate, 50 μM tetramethyl phenylene diamine (TMPD) and 630 μg/ml total chlorophyll (cuvette pathlength 1 mm). Redox potential of the reaction mixture about +200 mV. (B) (■–■) Same conditions as in fig.2A, but under background illumination to cause P700 photo-oxidation; (▲–▲) same as above, plus additional 10 μM methyl viologen. (C) (●–●) anaerobic reaction mixture containing all above reagents plus 50 μM of the low-potential viologens reduced by dithionite to −625 mV; (○–○) same as above plus background illumination to cause $A_2^{\cdot-}$ accumulation. For ms measurements, the 710 nm dye-laser excitation pulse of 300 ns duration was used (see [2]).

in conjunction with a single-beam spectrometer [12]. ΔA induced by continuous illumination were measured in a dual-wavelength spectrophotometer [13].

3. Results

Figure 2A shows the kinetics of ΔA $\Delta A_{694.3}$ in TSF-I particles posed at +200 mV and 20°C. During the instrumental resolution time of 60 ps a bleaching at 694.3 nm is observed, the amplitude of which is 2-fold greater than that induced by continuous illumination (dashed line). The decay from the maximum level to the level of steady-state bleaching has a lifetime of ~200 ps. When P700 was maintained in the oxidized state by continuous background illumination, the absorbance changes induced by a ps pulse were decreased 10-fold (fig.2B).

At −625 mV, when the bound iron–sulfur proteins are in the reduced state [14], ps excitation produces the same absorbance decrease (as in fig.2A), but the subsequent decay has an initial lifetime of 10 ns (fig.2C), and does not appear to be altered by background illumination (fig.2C). Under these conditions, the ΔA decay extends into the ms domain (fig.2C), and with decreasing temperature into the ms range [2]. The spectrum of these ΔA at 5 K, where their lifetime is 1.3 ms, is shown in fig.3A. In the red region only an intense bleaching of a 700 nm band is observed, while in the blue region a bleaching at 425 nm and at 450 nm is observed. New narrow bands at 480 nm and 670 nm are developed which cannot be attributed to P700 oxidation [2,3].

4. Discussion

The principal feature of the ΔA in TSF-I particles reported here (fig.2A) can only be accounted for by photochemical activity in the photosystem-I reaction centers, since maintenance of P700 in the inactive, oxidized state by supplementary illumination eliminated the ΔA almost entirely (fig.2B). We can rule out the possibility that these ΔA represent formation of an excited (singlet) P700* in 60 ps, followed by its

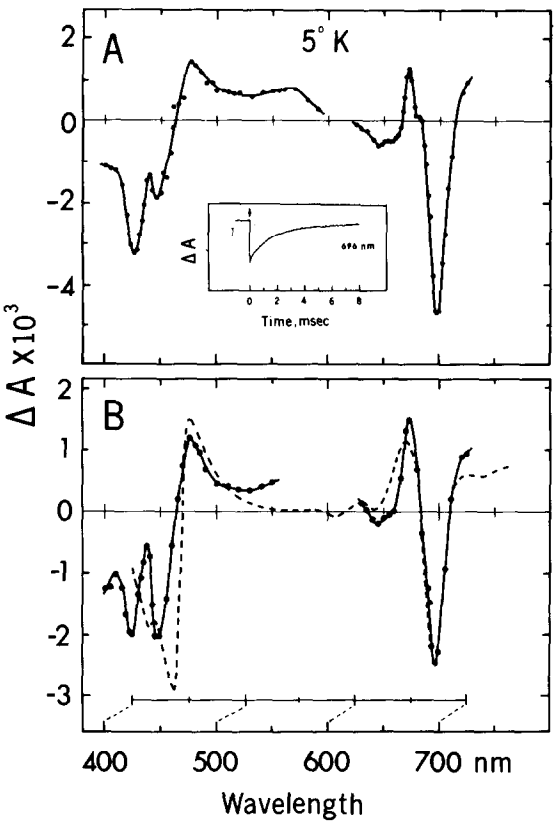

Fig.3. (A) Spectrum of light-induced ΔA with a lifetime of 1.3 ms measured in TSF-I particles poised at −625 mV at 5 K. The reaction mixture was the same as in fig.2C, except the chlorophyll was at 126 μg/ml (cuvette pathlength 1 mm). The spectrum was measured with a single-beam spectrometer, using 710 nm dye-laser excitation pulses for the 400–698 nm region, and 590 nm dye-laser pulses for the 698–725 nm region. The insert shows kinetics of ΔA_{696} induced by 710 nm laser excitation. (B) Solid curve: the result of subtraction of the ($P700^+$–P700) spectrum (fig.1A in [2]) from the spectrum in fig.3A, assuming that 50% of the bleaching at 700 nm is contributed by P700 photo-oxidation. The dashed curve is a retrace of the spectrum of ($Chl^{\cdot-}$–Chl) in solution taken from [8].

conversion to $P700^+$ in 200 ps, as this would imply a substantial molar A_{694} for $P700^+$ [2], and a much longer lifetime for the excited state of chlorophyll-*a* dimer (P700) than in [15]. It seems more likely, therefore, that light induces in TSF-I particles a rapid electron transfer (<60 ps) from P700 to some other chlorophyll species, probably also a dimer of chlorophyll *a* [2,3], both absorbing strongly around 694 nm, and that the anion radical of this dimer then donates an electron to an iron–sulfur protein in 200 ps.

At low redox potentials, the 10 ns decay apparently reflects recombination between $P700^+$ and the chlorophyll anion radical since the iron–sulfur proteins (P430) are now fully reduced in the dark, and the initial lifetime (10 ns) is not significantly altered when A_2 is maintained in its reduced state by the addition of background illumination (fig.2C) [2,3]. This recombination process seems to account for the ΔA in the ms domain also (fig.2C) since the corresponding ΔA at 5 K (1.3 ms) is, under similar conditions, kinetically the same as that of an EPR signal which is presumably the sum of cation and anion radical signals since its *g* value precludes its assignment to $P700^{\cdot+}$ alone [2]. It may be suggested that the 10 ns decay component reflects recombination in the ion–radical pair [$P700^{\cdot+}$·$(Chl)_2^{\cdot-}$] as well as interconversion into a state with some triplet character whose recombination time is 3 μs at room temperature (and 1.3 ms at 5 K).

If these assumptions are correct, we would expect the difference spectrum measured at −625 mV and 5 K (fig.3A) to consist of two overlapping spectra, one due to the formation of $P700^+$ and the other to the formation of a chlorophyll anion radical. Figure 3B shows the result of subtracting the spectrum of $P700^+$ formation [2] from the spectrum in fig.3A, assuming that 50% of the bleaching at 694 nm is due to P700 photo-oxidation (fig.2A). This net difference spectrum appears very similar to that for the formation of chlorophyll-*a* anion radical [8] retraced in fig.3B by the dashed line but shifted by 25 nm to the red to coincide with the spectrum obtained with TSF-I particles is dimeric, since the bleachings at 425 nm, ing that the chlorophyll-*a* acceptor in the TSF-I particles is dimeric, since the bleaching at 425 nm, 450 nm and 700 nm (fig.3B) coincide with those found for chlorophyll-*a* dimer in solution [9].

While A_1 can be identified on spectral and kinetic grounds as a chlorophyll-*a* dimer whose reduction is closely linked to photo-oxidation of P700, the role of A_2 (X) in the re-oxidation of $(Chl\text{-}a)_2^{\cdot-}$ is not yet clear, since its oxidation kinetics was not significantly altered by prior reduction of A_2 (fig.2C). Further work is planned to clarify this question.

It is of interest to note the resemblance between

the electron transfer sequence in TSF-I reaction centers deduced from our kinetic and spectral measurements and that of the bacterial reaction center: Electron transfer from the intermediary electron carrier, bacteriopheophytin, to the iron–ubiquinone complex occurs in about 250 ps [16–18], and recombination between the bacteriopheophytin anion radical and the primary electron donor, $P870^{+}$, in 6–10 ns [19].

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