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Kinetic studies on the function of A_1 in the photosystem I reaction center

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Flash absorption measurements with nanosecond time resolution were conducted in the near-ultraviolet with subchloroplast particles enriched in photosystem I, at 6°C. Around 370 nm, an absorption increase decays biphasically, with a fast phase, $t_{1/2} = 15 \pm 5$ ns. Control experiments show that this rapid phase is due to the reoxidation of A_1 (presumably phylloquinone), which thus appears to function as an electron carrier in PS I at physiological temperature. The nanosecond kinetic phase is not altered if the iron-sulfur centers F_A and F_B are kept reduced.

Photosystem I; Reaction center; Flash absorption spectroscopy; Electron transfer; Kinetics

1. INTRODUCTION

In the photosynthetic membrane of oxygenic organisms, excitation by light of the PS I reaction center induces a charge-separation process between an electron donor termed P-700 and a series of electron acceptors designated A_0 (presumably a specialized chlorophyll *a*), A_1 (presumably phylloquinone), and several iron-sulfur centers denoted as F_X, F_B, F_A. These electron acceptors are still poorly understood in terms of their chemical nature and structural or functional properties [1]. For example, our knowledge of the kinetics of forward electron transfer is restricted to a few values: around 30 ps for reoxidation of A_0^- [2] and less than 100 ns for reduction of one of the iron-sulfur centers [3]. A 170 ns transient in electron spin echo spectroscopy has not been precisely assigned [4].

The acceptor A_1 is presumably a phylloquinone (vitamin K_1), but the data are rather controversial, as discussed in the most recent publications in that field [5-8]. In a previous work, we have shown that extraction of phylloquinone from PS I induces a block of electron transfer away from A_0^-, which can be reversed by nearly stoichiometric addition of pure phylloquinone [9,10]. By flash absorption at low temperature, we have also shown that formation of the state P-700^+ A_1^- gives rise to absorption changes in the near-UV which decay with a half-time of 150 μs (at 10 K) and which can be interpreted in terms of the sum of contributions due to the oxidation of P-700 and to the formation of the phylloquinone radical anion [11]. Here, we use the same spectral properties to obtain the first kinetic information on the functioning of A_1 under physiological conditions.

2. MATERIALS AND METHODS

2.1. *Biological materials*

Subchloroplast particles enriched in PS I were prepared from spinach chloroplasts. Two kinds of particles were used: PS I-β with about 65 chlorophylls per P-700 [12] and PS I-110 with about 110 chlorophylls per P-700 [13]. For measurements at 6°C, about 100 μl of a stock solution was diluted in 2.5 ml of 50 mM Tris (pH 8.0), with 60 μl deoxycholate (10% w/w, in water), 10 μl of 100 mM Na ascorbate and 3 μl of 10 mM DCIP, unless otherwise indicated.

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Abbreviations: PS I, photosystem I; DCIP, dichlorophenolindophenol

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2.2. *Flash absorption*

The cuvette was excited by short flashes (30 ps, 532 nm; energy, about 5 mJ) provided at 0.2 Hz by a picosecond YAG laser (Quantel, type YG 401 SP) equipped with a light frequency doubler. The measuring light was provided by a xenon flash (USSI, type 1 CP-n) in which was discharged a bank of capacitors and inductances (voltage, 780 V; electrical energy, 9 J) arranged so as to provide a nearly rectangular profile for the light emission in the 10 μs time range. The measuring light was filtered before the cuvette with an interference filter at the studied wavelength, and focused through the cuvette onto a photomultiplier (ITT, type F4102). The photomultiplier output, loaded with 50 Ω, fed a Tektronix R-7912 digitizer equipped with a 7A13 differential amplifier. Before the cuvette, a piece of glass deflected part of the measuring light onto a reference photomultiplier, the output of which was amplified with a bandwidth of 0.3 MHz and connected to one input of the 7A13 amplifier.

The sample cuvette was usually cooled to 6°C. In some experiments we used a cryostat in which the cuvette (optical path 2 mm) was cooled to 10 K with helium gas.

The laser flash was synchronized so as to fire on the flat top of the xenon flash. From the signal obtained with N laser flashes ($N = 20$–640) with the xenon flash, we subtracted the same number of signals without xenon flash, in order to correct for the laser flash artefact (generally small) and for the baseline of the digitizer. In the configuration used, absorption transients could be measured in the time domain 5–500 ns after the excitation flash. Absorption changes at 820 nm were measured as in [9] with set-ups providing nanosecond or microsecond resolution.

3. RESULTS

From previous low-temperature studies [11], it was apparent that the 360–390 nm spectral region is the most favorable for kinetic studies on A_1^-. We thus measured the absorption transients induced by a flash, at 370 nm, in enriched PS I particles (fig.1). The absorption rises immediately and decays biphasically in the 200 ns time range: a fast phase with $t_{1/2} = 15 \pm 5$ ns, and a much slower phase. These data in the UV have a poor signal/noise ratio; this is essentially due to the basic reasons of a large time bandwidth, weak measuring light and small signal size. A few control experiments permit elucidation of the origin of the fast phase of decay. Firstly, addition of 0.1 mM ferricyanide, which maintains P-700 in the oxidized state, completely suppresses the ΔA (fig.1) which can thus be associated with PS I photochemistry. Addition of dithionite, under conditions which reduce the iron-sulfur centers F_A and F_B, as shown by the kinetics of ΔA at 820 nm (fig.2; [14]), does not significantly modify the ΔA at 370 nm which can thus be associated with primary electron-transfer steps not involving these iron-sulfur centers. Absorption measurements were also performed at 820 nm with nanosecond time resolution, under mildly reducing conditions with the same PS I particles and the same conditions for laser excitation (fig.1). The absorption increases immediately and does not decay significantly in the 100 ns time range: this shows that the fast phase at 370 nm cannot be associated either with re-reduction of P-700$^+$ (e.g. by a back-reaction with A_0^-) or with decay of the chlorophyll *a* triplet state. If we accept a simple scheme of electron transfer in PS I, with the species P-700, A_0, A_1 and F_X arranged sequentially, it appears that our results are only consistent with the 15 ns phase being associated with electron transfer from A_0^- to A_1, or from A_1^- to F_X. The first hypothesis can be excluded because A_0^- is reoxidized in the subnanosecond range [2] and also because we do not observe at 820 nm any oxidation decrease indicative of A_0^- reoxidation. We thus propose that the 15 ns decay is due to the reoxidation of A_1^- which reduces the next electron acceptor, presumably F_X.

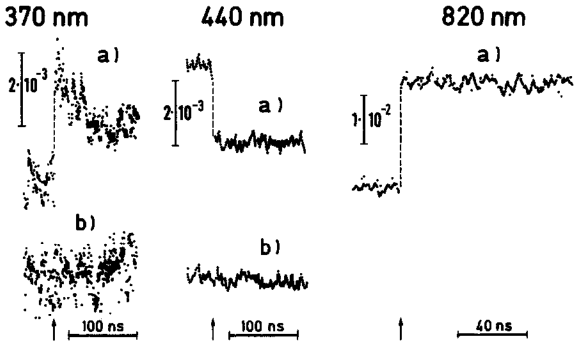

Fig.1. Nanosecond absorption changes induced by a laser flash in PS I-β particles, at 6°C. Addition of 0.1 mM ferricyanide (lower traces at 370 and 440 nm) and further addition of ascorbate and DCIP (other traces). Chlorophyll concentration: 0.017 mM at 370 nm (average of 160 flashes), 0.008 mM at 440 nm (40 flashes), 0.150 mM at 820 nm (40 flashes).

Flash absorption measurements were performed at other wavelengths in the UV and the blue, in order to check whether the signals were consistent with published difference spectra for (P-700$^+$, A_1^-) and for P-430$^-$ (supposing that F_X and F_A or F_B will have similar spectra [3,11]. The signal shape was similar at 350, 370, 380 and 390 nm (the wave-

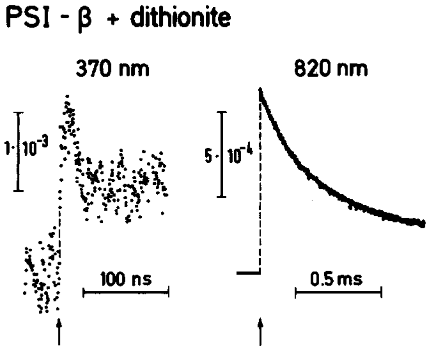

Fig.2. Absorption changes induced in a cuvette with PS I-β particles (chlorophyll, 0.011 mM) with addition of 1.2 mg/ml of Na dithionite (buffer: 100 mM glycine, pH 10). Measurement at 370 nm (average of 80 flashes; nanosecond apparatus) or 820 nm (average of 4 flashes; microsecond apparatus).

lengths for which we had filters available); the signal is maximum at 370–380 nm. In the blue the kinetics were different. At 440 nm (fig.1) and 430 nm, a bleaching stays constant within 200 ns, and is thus attributed to P-700 oxidation which would dominate absorption transients due to A_1 and the subsequent electron acceptor. Above 490 nm, the absorption change is positive and remains constant; it is also attributed to P-700 oxidation. Some small positive fast transients were present in the range 450–480 nm, but the poor signal/noise ratio precludes any quantitative description.

Some experiments were performed at low temperature (fig.3), the rationale being that any fast phase due to the reoxidation of A_1^- should disappear, since electron transfer from A_1 to F_X is largely blocked and A_1^- is reoxidized essentially by a 150 µs back-reaction with P-700$^+$ [1,11]. For this experiment we used D-144 PS I particles, since in PS I-β particles the low-temperature ΔA values are dominated by the P-700 triplet state (Sétif, P., unpublished). With D-144 particles the ΔA at 370 nm at 6°C includes a fast decay ($t_{1/2}$ = 10–15 ns), similar to that observed with PS I-β particles. At 10 K the absorption increase does not decay within 200 ns, in agreement with the hypothesis concerning the origin of the signal.

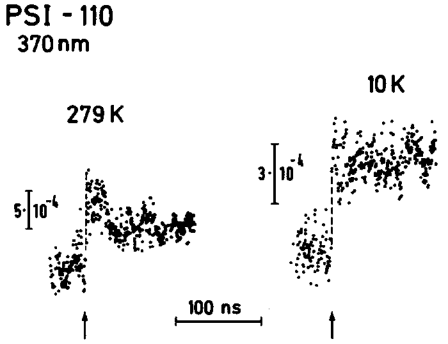

Fig.3. Absorption changes induced by a laser flash in cuvettes with D-144 PS I particles, at 370 nm, with addition of ascorbate and DCIP. Left trace: cuvette at 6°C; optical path, 10 mm; 0.014 mM chlorophyll; average of 600 flashes. Right trace: cuvette at 10 K; optical path, 2 mm; addition of 55% (v/v) glycerol; 0.061 mM chlorophyll; average of 640 flashes.

4. CONCLUSIONS

The present data are best interpreted by supposing that, in PS I, the secondary acceptor A_1 is rapidly photoreduced by a flash and reoxidized by forward electron transfer in the 20 ns time range at physiological temperature. Our previous spectral data at low temperature and extraction-reconstitution experiments were strong arguments for identifying A_1 with a phylloquinone, in agreement with experiments and proposals from other laboratories. However, the involvement of phylloquinone in PS I electron transfer at physiological temperatures has not yet been directly demonstrated. Our present data are a new contribution in that respect. The electron acceptor from A_1^- is probably F_X, as shown by the experiment of fig.2 where F_A and F_B were reduced by dithionite. A 15 ns half-time for the transfer from A_1^- to F_X is consistent with previous measurements of forward electron transfer in PS I: subnanosecond transfer from A_0^- to A_1 [2], about 170 ns for the transfer to the F_A-F_B pair [4] and less than 100 ns for the reduction of P-430 in a spectroscopic determination which might have measured the reduction of F_X as well [3].

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