SPECTRAL CHARACTERIZATION OF THE INTERMEDIATE ELECTRON ACCEPTOR (A_1) OF PHOTOSYSTEM I

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1. Introduction

Recent studies of chloroplast photosystem I have indicated the presence of an intermediate electron acceptor (A_1) which accepts electrons from the reaction center chlorophyll, P700, before they are transferred to more stable electron acceptors (A_2 and bound iron–sulfur centers A and B) [1–7]. The chemical identity of A_1 has been considered in recent publications: Friesner et al. [8] first suggested chlorophyll might act as this acceptor; and Shuvalov et al. [5] obtained evidence for a chlorophyll *a* dimer as the acceptor. Heathcote et al. [9] concluded on the basis of electron paramagnetic resonance (EPR) studies that a monomer of either chlorophyll or pheophytin could be the acceptor. We have obtained evidence for the function of either a chlorophyll or a pheophytin monomer as A_1 based on flash-induced optical studies and low-temperature EPR characterization of components in a photosystem I reaction center complex [10].

One complicating factor in studying A_1 has been that the conditions necessary to observe spectral changes of this carrier are difficult to obtain. Shuvalov et al. [5] used strongly reducing conditions where secondary electron acceptors, such as the bound iron–sulfur centers, are reduced prior to illumination while a different approach has been to use an electrophoretically isolated photosystem I preparation made with the detergent, sodium dodecyl sulfate (SDS) [2,10]; this preparation is devoid of secondary electron acceptors but still contains A_1. Here we report a convenient method for the preparation of photosystem I fragments which lack the secondary electron acceptors of photosystem I but retain a functional photosystem I reaction center in that P700 and A_1 are present. We have used this preparation for characterization of the flash-induced ΔA associated with the reduction of the intermediate electron acceptor.

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2. Materials and methods

Photosystem I fragments were prepared by a modification of the procedure in [11] using lauryl dimethylamine oxide (LDAO) treatment of digitonin chloroplast fragments. The LDAO-treated material is passed over a 2.5 × 90 cm Sephacryl S-200 column equilibrated with 50 mM Tris–HCl buffer (pH 7.8) + 1% Triton X-100. The first chlorophyll-containing fractions contain the highly enriched photosystem I fragments with a P700/chlorophyll ratio of 1:40. The properties of these fragments were identical to those in [11]. Freshly prepared fragments were heated at 60°C for 5 min and then centrifuged for 5 min at 30 000 × *g* to remove a small amount of insoluble material.

The apparatus for flash-kinetic spectrophotometry has been detailed in [10] and employs a Phase-R model 1100 flash-lamp pumped dye laser as the actinic light source. Broad band red light (650–680 nm) was used to activate the samples. The time-response of the apparatus was ~3 μs, and the light-on kinetics which we observed are instrument-limited.

3. Results

We have based our experimental procedure for the removal of the secondary electron acceptors from photosystem I on the finding in [12] that the appear-

ance of delayed luminescence correlated with the disappearance of secondary electron acceptors (measured as the P430 ΔA) in heat-treated photosystem I fragments. Accordingly, one might expect heat-treated photosystem I fragments to show P700 photooxidation and A_1 reduction, followed by a rapid back-reaction, because such preparations would lack the more stable electron acceptors.

According to [12], heat treatment at 60–65°C was sufficient to destroy P430. Such a treatment of our photosystem I fragments results in slight changes in the absorption spectrum of the preparation: a blue shift from 675–668 nm occurred but other spectral changes were minimal. Most significantly, either before or after heating, our preparations had negligible $A_{520-540}$ where oxidized pheophytin would be expected to absorb.

Flash-induced studies of chloroplasts [4] as well as photosystem I fragments [1–3] have shown that the back-reaction between $P700^+$ and A_1^- has a half-time of ~5 μs at 20°C. We have observed a similar half-time in the SDS–photosystem I reaction center complex devoid of secondary electron acceptors [10]. As shown in fig.1A, the flash-activated kinetic response of the control preparation before heating, monitored at 430 nm, shows an instrument-limited absorbance decrease followed by a slow decay. Kinetic studies at

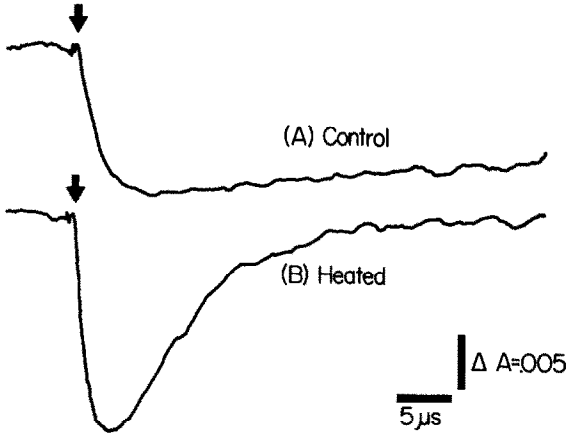

Fig.1. Kinetics at 430 nm of the flash-induced ΔA at 25°C in control and heat-treated photosystem I fragments. The reaction mixture contained 25 mM Tris–HCl buffer (pH 7.8), 10 mM ascorbate, 10 μM tetramethylphenylenediamine, 10 μM methyl viologen and chloroplast fragments at 6 μg chlorophyll/ml. (A) Control fragments. (B) Heat-treated fragments. In (B), methyl viologen and tetramethylphenylenediamine were omitted from the reaction mixture. Each trace is the result of a single laser flash.

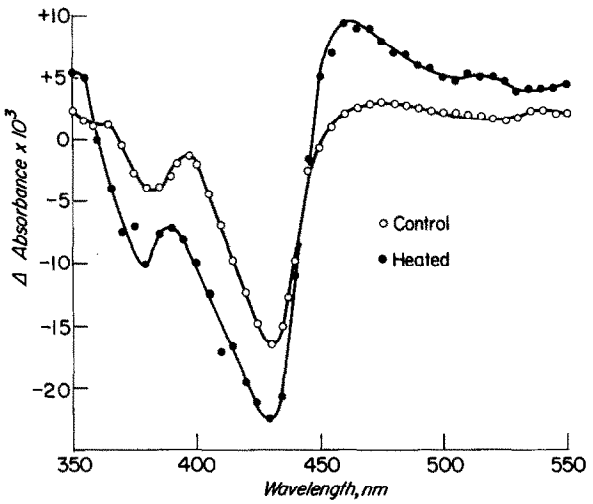

Fig.2. Light-minus-dark difference spectrum of the flash-induced ΔA in control and heat-treated photosystem I fragments. Points were obtained as in fig.1, most from a single laser flash, and a few the result of 2–6 averaged flashes/point. A bandwidth of 5 nm was used for all points.

a slower response time have shown a biphasic decay, the slower component having a half-time of ~0.5 ms under these conditions. The kinetic response at 430 nm of the preparation heated at 60°C for 5 min is shown in fig.1B, and a rapid decay of the light-induced absorbance decrease is observed: the half-time of ~7 μs is comparable to that observed in [2,10] for conditions where this reaction represents the $A_1^- + P700^+$ back-reaction.

The spectra of the flash-induced ΔA in the region from 350–550 nm are shown in fig.2. The spectrum of the control sample was obtained under conditions where electrons in the primary electron acceptor complex are transferred to oxygen and the resulting spectrum represents that from P700. This spectrum is characterized by a relatively narrow band in the blue region, centered at 430 nm, and is similar to the spectrum of P700 reported in this spectral region [5,12,13]. The spectrum obtained after heat treatment shows significant differences from that of the control: the amplitude is increased, the peak in the blue region is considerably broader and there is a large A_{380} decrease as well as an A_{470} increase. This spectrum would then originate from a combination of the spectra of $P700^+$ and A_1^-. Subtraction of the $P700^+$ spectrum from that obtained in the heat-treated sample yields a spectrum for A_1^- with A_{375} and A_{410} decreases and an A_{460} increase.

4. Discussion

Our results indicate that heat-treatment of photosystem I fragments produces a preparation with flash-induced kinetics characteristic of the presence of only one electron acceptor (A_1) for P700. These findings imply that secondary electron acceptors are heat-labile and are consistent with the finding in [12] that the appearance of delayed luminescence in photosystem I fragments paralleled the inactivation of the secondary electron acceptors. The delayed luminescence would then originate as a result of the back-reaction between A_1^- and $P700^+$.

The spectral changes associated with the reduction of chlorophyll and pheophytin to the respective anion radicals have been studied in detail in model systems [14]. The spectral changes for both components are similar from 350–500 nm, showing absorbance increases below 350 nm and at ~470 nm and a large bleaching in the Soret band region. The most significant differences in absorbance between reduced chlorophyll *a* and reduced pheophytin *a* occur between 500–540 nm since pheophytin exhibits a doublet which is bleached on reduction in this region [14]. Our spectral analysis of the heated photosystem I fragments are most consistent with a chlorophyll *a* molecule functioning as A_1 since these preparations contain little or no pheophytin, and no significant $\Delta A_{500-540}$ were observed.

We have concluded [10] on the basis of EPR measurements of an SDS–photosystem I reaction center complex at cryogenic temperatures that the intermediate electron acceptor of photosystem I is a pigment (chlorophyll or pheophytin) monomer molecule. On the basis of these studies, we would argue that a chlorophyll *a* monomer serves this role. The redox properties of the chlorophyll *a* anion radical are consistent with this proposed function in the photosystem I electron acceptor complex [14].

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