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# EVIDENCE THAT THE INTERMEDIATE ELECTRON ACCEPTOR, $A_2$, IN PHOTOSYSTEM I IS A BOUND IRON-SULFUR PROTEIN

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## Summary

Absorption changes accompanying the formation of light-induced *P*-700$^+$ were investigated in a highly enriched Photosystem I preparation where an intermediate electron acceptor preceding *P*-430 could be detected. In an enriched Photosystem I particle, light-induced reversible absorption changes observed at 700 nm in the presence of dithionite resembled those previously seen at 703 nm and 820 nm [9], thus indicating the presence of a backreaction between *P*-700$^+$ and $A_2^-$. After this same Photosystem I particle was treated to denature the bound iron-sulfur centers, the photochemical changes that could be attributed to *P*-700 $\rightleftharpoons$ $A_2$ were completely lost. These results provide evidence that the intermediate electron acceptor, $A_2$, is a bound iron-sulfur protein. Additional studies in the 400—500 nm region with Photosystem I particles prepared by sonication indicate that the spectrum of $A_2$ is different from that of *P*-430.

Identification of the components comprising the primary electron acceptor complex of Photosystem I has proceeded largely through the successful application of electron spin resonance and optical techniques. Electron spin resonance techniques have uncovered the existence of three components functioning on the reducing side of Photosystem I: two membrane-bound iron-sulfur centers (ESR Centers A and B) [1,2] and one, as yet unidentified ESR component denoted as "X" [3,4]. Until very recently, optical techniques revealed only one component, *P*-430 [5,6].

We have previously described a method [7] to denature the bound iron-sulfur proteins in a purified Photosystem I particle without destroying the primary electron donor, *P*-700 (as indicated by the ascorbate-minus-ferricyanide difference spectrum). In the course of denaturation, we observed a

Abbreviations: DPIP, 2,6-dichlorophenolindophenol.

strictly linear relation between the remaining bound iron-sulfur centers and the flash-induced *P*-700 [8]. However, the time resolution of our instrument only allowed resolution of events occurring >1 ms after the flash. Consequently, we could not exclude the possibility of *P*-700-mediated charge separation followed by recombination in the sub-millisecond time range. Recently, it was reported [9] that in the presence of a powerful reductant, the normal, long-lived charge separation was replaced by one with a 200-$\mu$s half-time. This fast transient has been interpreted as the backreaction between *P*-700$^+$ and an electron acceptor more primary than *P*-430, named $A_2$.

The purpose of the present study was to search for the intermediate acceptor, $A_2$, in a highly enriched Photosystem I particle (spinach A-III) and in one lacking the functional iron-sulfur centers.

The enriched Photosystem I preparations used in this study consisted of spinach A-III particles isolated from detergent-solubilized membranes according to the procedure of Golbeck et al. [7]. The buffer used throughout the isolation was Tris-HCl (0.05 M, pH 8.8) containing 1% Triton X-100 and 0.2 M KCl. Inactivated System I particles (lacking functional iron-sulfur centers) were prepared from A-III particles according to the method described in ref. 7. Absorption changes were measured using a single-beam spectrophotometer capable of 50-$\mu$s time resolution. The wavelength of the measuring beam was selected either with interference filters as described previously [8] or with a monochromator. A xenon flash, passed through suitable colored filters, provided actinic illumination. A photodiode served as the detector and, after suitable amplification, the signals were recorded using a transient recorder (Fabri-Tek 1052 or Biomation 802).

When active Photosystem I particles are preincubated with sodium ascorbate and DPIP and illuminated with a flash, the oxidation of *P*-700 is followed by a backreaction with *P*-430$^-$, resulting in a partial re-reduction with a 10-ms half-time [8]. In this plastocyanin-depleted particle, the recovery of the remaining *P*-700$^+$ is slow and depends on the concentration of the artificial electron-donor couple, ascorbate-DPIP. When the oxidation of *P*-430$^-$ is accelerated by electron acceptors, such as methyl viologen or spinach ferredoxin, the backreaction is inhibited, and the entire *P*-700$^+$ reduction becomes slow [8].

Fig. 1 shows the photooxidation of *P*-700 after consecutive flashes in a Photosystem I particle incubated with dithionite at pH = 10. The first flash induces an absorbance decrease at 700 nm which decays with a half-time of 10 ms. After a few flashes, however, a large acceleration in the *P*-700$^+$ decay is obtained, stabilizing in the rapid decay following the fourth or fifth flash. The half-time of the rapid decay is estimated to be 200—300 $\mu$s, or similar to those seen by Sauer et al. [9] following the addition of dithionite to a TSF-1 particle. Accordingly, the decay can be ascribed to a reduced intermediate acceptor, $A_2^-$, backreacting with *P*-700$^+$. Note that no background illumination was used prior to the first flash; multiple flashes were sufficient to induce the fast decay.

We next studied a Photosystem I A-III particle that was modified so that nearly all of its iron-sulfur centers were denatured. The remaining iron-sulfur protein (estimated as labile sulfide) and *P*-700 (determined by ascorbate-minus-

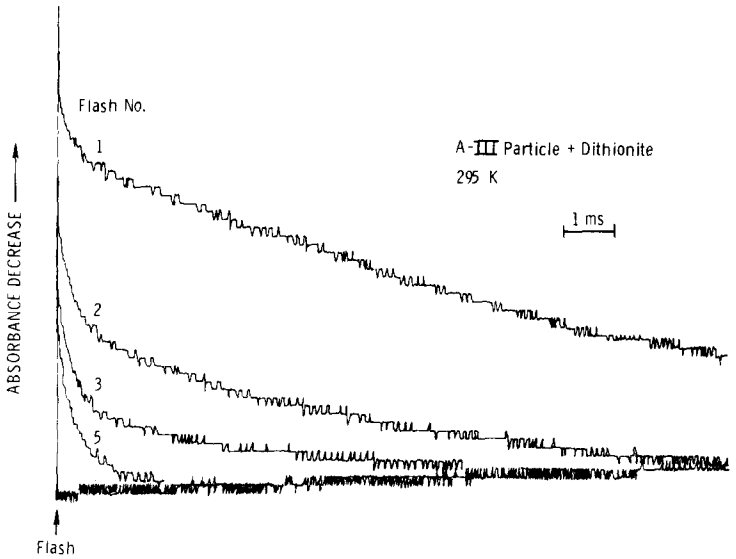


**Fig. 1. Absorption changes at 700 nm in a spinach A-III particle after the addition of a few grains of dithionite. The particles were suspended in glycine buffer (0.05 M, pH 10.0) containing 1% Triton X-100 and 0.2 M KCl and illuminated with a flash the number of times indicated. Baseline was determined using the measuring beam only prior to the first flash. The signal was stored in a Fabri-Tek 1052 prior to readout on a Hewlett-Packard 7004B X-Y Recorder.**

ferricyanide difference spectroscopy and photochemically 2 ms after a saturating flash) are shown in Table I for the control and inactivated particles. We have previously shown [8] that particles depleted of non-heme iron and labile sulfide lack electron spin resonance Centers A and B after chemical or photochemical reduction. Since *P*-700 remains almost entirely intact (as assayed by the chemical difference spectrum), the inability of the particle to exhibit photooxidation must be due to a lesion or multiple lesions on the acceptor side of the photoact. We found that, in accord with their small complement of remaining iron-sulfur centers, these particles showed very little photo-induced *P*-700 oxidation when monitored several ms after a flash. We also found (monitoring at 700 nm) that the sub-ms kinetics described above and in ref. 9 were not observed in these depleted particles, under conditions where the transient could be observed in the native preparation. This is

TABLE I

LABILE SULFIDE AND *P*-700 CONTENT OF CONTROL AND INACTIVATED SPINACH A-III PARTICLES

Chloroplast A-III particles were incubated in 4 M urea and 5 mM ferricyanide for 4 h and then passed over Bio-Gel P-4 as described in Materials and Methods of ref. 7. The chlorophyll content was 75 μg/ml.

| Sample | $P\text{-}700^{CD}$* (μM) | $P\text{-}700^{PC}$** (% control) | Acid-labile sulfide (μM) |
|---|---|---|---|
| Control | 2.275 | 100 | 23.80 |
| Inactivated | 2.010 | 16 | 3.14 |

*$P\text{-}700^{CD}$ refers to the ascorbate-minus-ferricyanide chemical difference spectrum.
**$P\text{-}700^{PC}$ was measured photochemically 2 ms after a saturating flash, in the presence of 0.1 mM methyl viologen.

readily explained if we assume that the acceptor ($A_2$) involved in the 200-$\mu$s transient is itself an iron-sulfur protein.

In detergent-treated particles, flash-induced triplet formation of bulk chlorophyll occurs [10]. Although this caused little interference at 700 nm, triplet decay prevented us from using detergent-treated particles to study $P\text{-}700^+ \rightleftharpoons A_2^-$ backreaction in other parts of the spectrum. To study spectral characteristics in the blue, flash-induced absorption changes at 433, 448, and 488 nm were monitored using sonicated chloroplast fragments prepared according to the method of Jacobi [11].

Fig. 2a and b shows absorption changes in the presence of ascorbate and under conditions optimal for sub-ms decay: the presence of dithionite and weak, red background illumination. In Fig. 2b, the half-time of the decay, which is most accurately observed at 433 nm, is about 200 $\mu$s. We found that the flash intensity required for light-saturation of this signal was similar to that needed for *P*-700 under normal (slowly recovering) conditions, implying that the photochemical cross section and efficiency of exciton transfer are the same for *P*-700 under both sets of conditions. The flash-induced changes shown in Fig. 2a and b are, to a large extent, similar, and we have concluded that the photooxidation of *P*-700 dominates the spectral changes in both cases (cf. ref. 12). However, the spectral response of *P*-700 and its reaction partner in the 200-$\mu$s decay (Fig. 2b) was not completely identical to that of *P*-700 and *P*-430 (the partner in Fig. 2a); the divergence shows up clearly around 448 nm. In Fig. 2a, the absorption increase at that wavelength is due

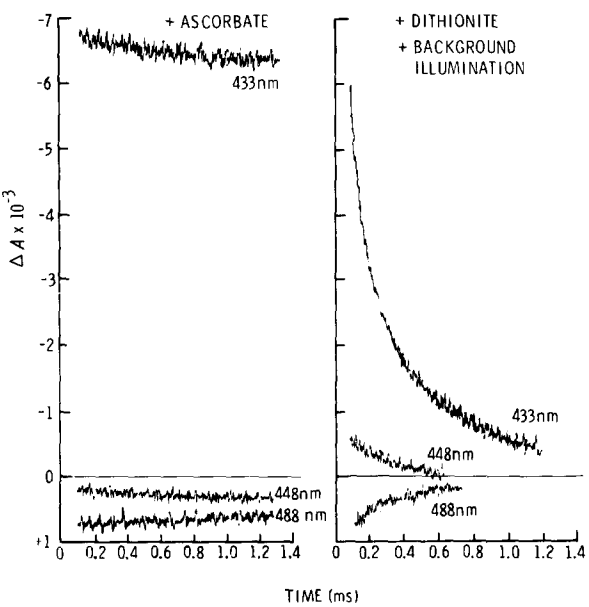


**Fig. 2. Flash-induced absorbance changes in the presence of ascorbate-DPIP (left) and after the addition of dithionite plus background illumination (right) in a Photosystem I particle prepared by sonication. The absorbance change occurring between 0 and 0.1 ms is a flash artifact and is not shown for reasons of clarity. The signal was recorded using a Biomation Model 802 transient recorder.**

to combined $P\text{-}700^+$ and $P\text{-}430^-$; in Fig. 2b, the 200-$\mu$s transient reflects the decay of an absorbance decrease.

If $A_2$ is, indeed, a bound iron-sulfur protein, as this study suggests, it is uncertain which ESR component it represents. Even though $P$-430 can probably be identified with ESR Center A [13], we cannot determine from the available data whether the new optical component, $A_2$, represents ESR Center B or the newer component, "X". Our spectral studies show that while $A_2$ may be one of the membrane-bound iron-sulfur proteins, its spectrum is not completely identical to that of $P$-430.

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