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ELECTROCHEMICAL AND SPECTRO-KINETIC EVIDENCE FOR AN INTERMEDIATE ELECTRON ACCEPTOR IN PHOTOSYSTEM I*

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Summary

Absorption changes accompanying light-induced *P*-700$^+$ formation and its decay in the dark at 15 K in Photosystem-I particles poised at various redox potentials have been examined. In unpoised samples, the light-induced absorption change is practically irreversible. At increasingly negative potentials, an increasing fraction of the absorption change, proportional to the fraction of bound iron-sulfur protein chemically reduced, becomes reversible, and the titration curve has a midpoint potential of —530 mV (vs. normal hydrogen electrode). At —666 mV, the *P*-700 absorption change is 97% reversible. The total *P*-700-signal amplitude decreases over the same potential span and levels off at about 43% (to slightly over 50% at a substantially higher excitation intensity). These results provide additional support to previous suggestions of an existence of an intermediate electron acceptor located between the primary donor, *P*-700, and the more stable primary electron acceptor (*P*-430 or bound iron-sulfur protein).

In 1971, a spectral species, *P*-430, detected by flash-kinetic spectroscopy [1], and a membrane-bound iron-sulfur protein, detected by EPR spectroscopy [2], were separately proposed to be the primary electron acceptor of Photosystem I. Since then, redox titration of membrane-bound iron-sulfur proteins in Photosystem-I subchloroplasts on the one hand [3, 4], and the light-induced absorption changes accompanying *P*-700 photooxida-

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tion near liquid-nitrogen temperature on the other [5], led to the conclusion that one of the bound iron-sulfur proteins functions as the primary electron acceptor of Photosystem I. This identification is further supported by the parallel decay kinetics of photooxidized *P*-700$^+$ and photo-reduced iron-sulfur protein at various low temperatures [6—8]. The parallel kinetic decay was taken to represent the recombination between the two primary reactants in the dark period.

However, an anomaly had been noted in our earlier redox titration results, namely, a small (approx.10%), rapidly reversible *P*-700 signal persisted even at potentials sufficiently negative to insure complete reduction of all bound iron-sulfur proteins [5]. In the meantime, work from other laboratories [9, 10] on low-temperature EPR spectroscopy with Photosystem-I subcholoroplast particles suggested a possible existence of an intermediate electron acceptor located between *P*-700 and the more stable primary acceptor, the bound iron-sulfur protein.

In view of these developments, we recently reexamined the redox titration of the Photosystem-I particles by monitoring the light-induced formation of *P*-700$^+$ radicals by EPR spectroscopy at 90 and 15 K [11]. The EPR results obtained at 90 K agree with those spectro-kinetic measurements reported earlier [5]. For titrations monitored at 15 K, significant difference in several respects were observed. Throughout the course of reductive titration of bound iron-sulfur proteins, the amplitude of the light-induced EPR signal of *P*-700$^+$ remained constant, but the percentage of reversibility increased with decreasing potential. When all bound iron-sulfur proteins are reduced, the reaction is about 90% reversible. At potentials more negative than —700 mV, the amplitude of the fully reversible light-minus-dark signal also begins to diminish. These results were interpreted as to indicate the existence of an intermediate electron acceptor (hereafter designated as "I") between the primary donor, *P*-700, and the more stable* primary acceptor (*P*-430 or iron-sulfur protein). The decrease of the *P*-700$^+$ signal amplitude at more negative potentials suggests the beginning reduction of the intermediate acceptor, the midpoint of which was estimated to be near —730 mV [11].

This note reports new redox titrations of Photosystem-I particles monitored by light-induced absorption changes at 15 K. The results provide additional support to the conclusion derived from recent EPR studies [11].

The experimental setup is similar to that described in ref. 13, except precision quartz cuvettes with a pathlength of 1 mm (James F. Scanlon Co., Solvang, Calif.) were used in the titration and optical measurements. Because electrochemical reduction was difficult for a suspending medium containing 55% glycerol, 0.1 M dithionite was used as the titrant.

The sample being titrated was suspended in 0.1 M glycine buffer, pH 11.0, at a chlorophyll concentration of 150 μg/ml. The particles contain one *P*-700 per 45 chlorophyll molecules, and have an NADP-reduction activity of 1550 μmol$\cdot$mg^{-1} Chl$\cdot$h^{-1}. In addition to 1 mM ascorbate and 50 μM

*Meaning the first intermediate that can be stabilized for times of the order of milliseconds (also cf. ref. 12).

tetramethyl-*p*-phenylenediamine, only three redox mediators, methyl viologen, 1,1′-trimethylene-2,2′-dipyridylium dibromide and 1,1′-trimethylene-4,4′-dimethyl-2,2′-dipyridylium dibromide were used, all at 100 μM. Titration and sample transfer procedures have been described previously [3, 13, 14]. A dual-wavelength spectrophotometer which can automatically correct for fluorescence interference [15] was used; the light-induced absorption change was measured at 700 nm vs. 725 nm as the reference wavelength. The excitation light was isolated with one Corning 4-96 filter and three Schott BG-18 filters and 1.5 inches of water, and had an incident intensity of $3 \cdot 10^5$ ergs$\cdot$cm$^{-2}\cdot$s^{-1}. The photomultiplier was protected from the excitation light by Schott RG-8 and a broad-band interference filter with a peak transmission at 710 nm and a halfwidth of 30 nm (Baird Atomic).

The light-induced absorption change accompanying the photooxidation of *P*-700 at 15 K in the Photosystem-I particles containing ascorbate, tetramethyl-*p*-phenylenediamine and the viologen mediators prior to titration (apparent potential of the sample was about 0 mV, vs. normal hydrogen electrode) is similar to the first trace in Fig. 1. The light-induced absorption change is 97% irreversible at 15 K.

As the potential becomes more negative, a greater change in the percentage of decay and the initial amplitude becomes noticeable. At −486 mV (second trace in Fig. 1), noticeable decay is apparent and the total amplitude also begins to decrease. The remaining four traces show absorption-change signals in samples poised at potentials where an increasing fraction of the bound iron-sulfur proteins becomes reduced. At −629 mV, practically all bound iron-sulfur proteins are expected to be reduced [3] and the absorption change is mostly reversible (Fig. 1).

To facilitate further discussion, the total signal amplitude and the percentage of irreversible signal based on the original total signal are plotted vs. the potential in Fig. 2. The percentage of irreversible reaction is apparently inversely proportional to the fraction of iron-sulfur proteins reduced chemically. At the most negative potential, only 3% of the reaction is irreversible (not shown in Fig. 1). The midpoint potential of the plot of the irreversible fraction vs. the potential is at −530 mV (vs. normal hydrogen electrode), which is in good agreement with our previous EPR results [11]. The total *P*-700 signal amplitude decreases over the potential span in which the bound iron-sulfur protein is reduced, but levels off at about 43% at the more negative potentials (Fig. 2).

These results may be interpreted in a similar way as for the redox-titration results obtained by monitoring the light-induced *P*-700$^+$ formation at 15 K by EPR spectroscopy [11]. In the unpoised subchloroplasts or before the bound iron-sulfur protein is chemically reduced, photon absorption by Photosystem I leads to *P*-700 photooxidation and an electron transfer to an unidentified intermediate electron acceptor "I" and then to the stable primary acceptor, the bound iron-sulfur protein. Although no direct evidence is available on whether these acceptors are arranged in a parallel or series pathway relative to *P*-700, we are assuming the series model in our present considerations.

The electron ejected by *P*-700 presumably resides very briefly on "I"

and then transferred to the iron-sulfur protein with midpoint potential of −530 mV and only a small fraction (3%) returns to $P\text{-}700^+$. The back reaction between the iron-sulfur protein and $P\text{-}700^+$ is negligible at this temperature [6], probably due to the small Boltzmann energy available [16], and this results in a practically irreversible signal as shown by the first trace in Fig. 1.

As the iron-sulfur protein begins to be reduced, a lesser fraction of it remains available for accepting electrons from $I^{\cdot -}$. The increasing amount of dark decay indicates an increasing extent of recombination between $I^{\cdot -}$ and $P\text{-}700^+$. The relationship between the percentage of decay vs. the potential

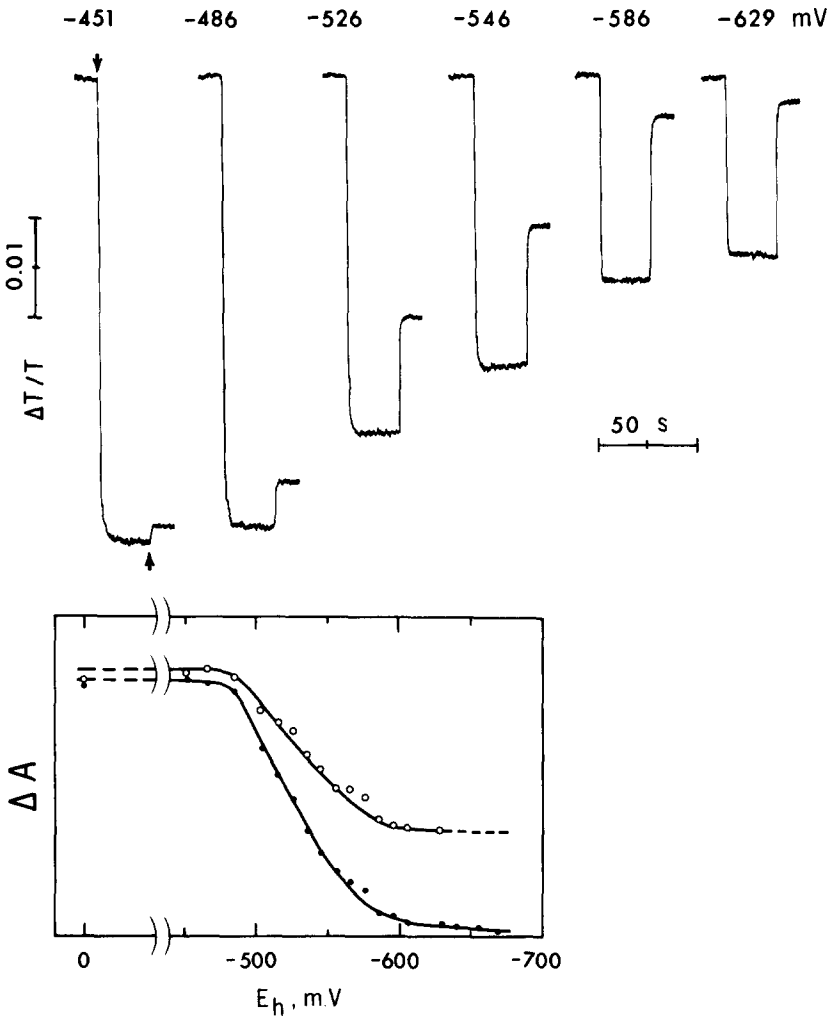

Fig. 1. Light-induced absorption changes at 15 K in samples poised at various potentials (indicated at top). Arrows indicate excitation light on and off. See text for other details.

Fig. 2. Plot of the total signal amplitude (○) and the irreversible fraction of the light-induced *P*-700 absorption change (based on the unattenuated signal obtained in the unpoised sample; cf. trace 1 in Fig. 1) (●) vs. the redox potential.

(Fig. 2) is thus an indirect representation of the percentage of the iron-sulfur protein present in the reduced state. These results, particularly with regard to the involvement of only the higher-potential iron-sulfur protein as the stable primary acceptor at low temperatures, are consistent with our previous findings [5, 11] as well as the findings of Bearden and Malkin [17].

The decrease in the total amplitude of the light-induced signal with increasingly negative potentials (Fig. 2) is at variance with previous EPR results also obtained at 15 K [11], which showed that the signals had a constant amplitude over the potential span down to −700 mV. The reason for this difference is not completely clear at present. Possibly, since the recombination between $P\text{-}700^+$ and $I^{\bullet -}$ is apparently very rapid even at 15 K, a high photon flux may be necessary to maintain an undiminished steady-state level. Thus we may assume that the attenuation of the total amplitude could be due to an inadequate photon flux. Some preliminary experiments with a substantially higher excitation intensity ($8.7 \cdot 10^5$ ergs$\cdot$cm$^{-2}\cdot$s^{-1}) brought the total amplitude to slightly over 50%. The resolution of this question requires further investigation. One possible means for circumventing this difficulty would be making measurements using low-temperature laser flash-kinetic spectroscopy, where sufficient excitation intensity and adequate time resolution may prevent the attenuation of the absorption-change signal due to rapid recombination of the primary reactants.

The nature of the rapidly reversible absorption change in samples poised at highly negative potentials has previously been determined to be that of *P*-700, either from the light-minus-dark difference spectrum or from EPR spectroscopy (ref. 11 and unpublished results), and not due to some other metastable state of chlorophyll, say, chlorophyll triplet. With sufficient time resolution, additional information regarding the recombination mechanism may also be revealed. With currently available time resolution, it is apparent the recombination is extremely rapid even at liquid-helium temperature. This suggests that the intermediate electron acceptor "I" must be in very close proximity to the donor molecule *P*-700.

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