

## BBA Report

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### Evidence for the identity of P430 of Photosystem I and chloroplast-bound iron–sulfur protein\*

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#### SUMMARY

The EPR spectrum of Photosystem-I subchloroplast particles, which had been pre-illuminated in the presence of methyl viologen, showed a large P700<sup>+</sup> signal whereas bound iron–sulfur proteins were not detected. This observation is consistent with a “one-way” electron discharge by the primary electron acceptor, P430<sup>−</sup>, subsequent to the primary photochemical charge separation, and an accumulation of photooxidized P700<sup>+</sup>. Subsequent illumination of the same sample at 77 °K did not change the EPR spectrum. However, if the pre-illuminated subchloroplast particles were allowed to recover at room temperature by standing in the dark for 10 min or by addition of a chemical reductant, subsequent illumination of the sample at 77 °K yielded an EPR spectrum consisting of signals due to both P700<sup>+</sup> and reduced iron–sulfur protein.

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Subsequent to the photochemical charge separation in Photosystem I, namely, the formation of the primary oxidant and reductant, there are five possible routes available for their disposition<sup>1</sup>. Three of these result in the return of the charged species to the uncharged state: by recombination of the charges, by a cyclic electron flow through an intermediate secondary electron carrier, or a non-cyclic electron flow by interaction with separate secondary acceptors and donors, respectively. Two other routes result in a temporary accumulation of either the primary oxidant, P700<sup>+</sup>, or the primary reductant, P430<sup>−</sup>, respectively, if either an efficient secondary acceptor or a donor but not both are present.

By means of optical spectroscopy, it was shown that when Photosystem-I sub-

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Abbreviation: TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

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chloroplast particles were pre-illuminated in the presence of an autooxidizable secondary electron acceptor only, subsequent flash illumination caused no transient absorption changes due to P700 photooxidation<sup>2</sup>. During pre-illumination, the primary reductant, P430<sup>-</sup>, presumably discharged its electron to the secondary acceptor such as methyl viologen, which was in turn autooxidized by air, and the net result was an accumulation of P700<sup>+</sup>. In fact, the recovery (re-reduction) of P700<sup>+</sup> was sufficiently slow that its difference spectrum could be easily measured from the difference between illuminated and unilluminated samples<sup>2</sup>. Unlike in the other four reaction routes (see above), spectroscopic detection of P430<sup>-</sup> is not practical because of the rapid discharge of electrons by this species. Of course, upon addition of a suitable electron donor such as *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) to the subchloroplast mixture containing methyl viologen, a photochemical charge separation followed by a non-cyclic electron flow involving both P700 and P430 can be immediately demonstrated.

In 1971, the primary electron acceptor of Photosystem I was identified in this laboratory<sup>3</sup> by optical spectroscopy and the spectral species was designated as "P430". About the same time, Malkin and Bearden<sup>4</sup> observed a ferredoxin-type EPR signal in illuminated chloroplasts at low temperatures and suggested that a membrane-bound ferredoxin is the primary electron acceptor of Photosystem I.

Our earlier spectroscopic evidence also suggested ferredoxin as one of the potential candidates for P430<sup>3,5,6</sup>. However, a definitive identification of P430 with ferredoxin was difficult because the absorption-band profile was slightly different from that of the soluble spinach ferredoxin and the magnitude of the difference extinction was also greater than that known for soluble spinach ferredoxin.

Thus the identification of P430 has remained an unsolved problem. This note presents results from our effort of correlating the behavior of P430 with that of bound ferredoxin by low-temperature EPR spectroscopy. This is attempted here in a system in which the reaction partner of P430 is trapped in the charged state.

Digitonin-fractionated Photosystem-I particles (D-144) prepared by the method of Boardman<sup>7</sup> were used. These particles are similar to the Triton-fractionated particles used in the earlier work<sup>2</sup> with respect to P700<sup>+</sup> accumulation as manifested by the optical changes. In Fig. 1, the two signals at left show that flashing of D-144 particles which had been pre-illuminated in the presence of methyl viologen did not produce light-induced absorption changes at 430 or 442 nm. The former wavelength encompasses changes due to both P700 photooxidation and P430 photoreduction, the latter wavelength is an isosbestic point for P700 and is thus due to P430 photoreduction alone. As shown earlier<sup>2</sup>, P700<sup>+</sup> can recover completely if the sample is allowed to stand in the dark for a sufficient amount of time (5–10 min) and to undergo another photochemical "one-way" electron discharge again, or the accumulated P700<sup>+</sup> can be rapidly restored to the uncharged state by adding TMPD to the pre-illuminated mixture. Subsequent flashing can then induce a non-cyclic electron flow. The two signals at the right in Fig. 1 were again measured at 430 and 442 nm after TMPD had been added to the pre-illuminated sample. The initial onset of absorption changes represents the photochemical charge separation,

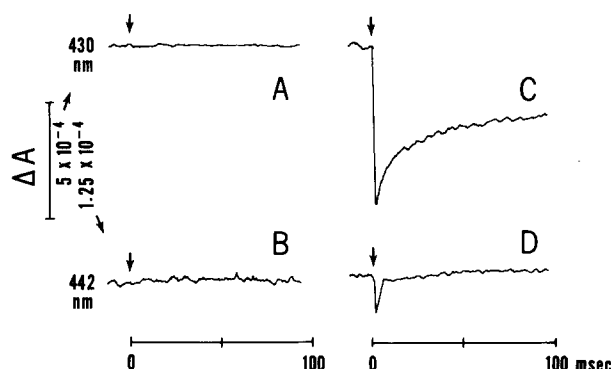


Fig. 1. Flash-induced absorption changes (A,B) in digitonin-fractionated Photosystem-I subchloroplast particles (chlorophyll concn, 1.04 mg/ml) in 0.1 M Tricine buffer, pH 8, pre-illuminated in the presence of methyl viologen (20  $\mu$ M). Pathlength of sample cuvette, 0.20 mm. The experimental conditions were the same as in ref. 2. The measuring wavelengths were 430 nm for A and 442 nm for B. Traces C and D are absorption changes in the same reaction mixture with ascorbate (0.5 mM) and TMPD (2  $\mu$ M) added. Traces A,C and B,D were averaged from 8 and 64 repetitive red actinic flashes, respectively.

and the dark decay involves interaction of  $P700^+$  and  $P430^-$  with the secondary electron donor (TMPD) and acceptor (methyl viologen), respectively<sup>1,2</sup>.

The premise of the EPR experiment was that D-144 particles pre-illuminated in the presence of methyl viologen should yield only an EPR signal for  $P700^+$  but no signal for the reduced bound ferredoxin, if indeed the latter is the primary electron acceptor and identical with  $P430$ ; rapid "one-way" electron discharge should then have returned the bound ferredoxin to the oxidized state. Furthermore, additional illumination of the frozen sample should produce no change in the EPR spectrum, since no new photochemical charge separation can take place when all  $P700$  is accumulated in the oxidized state. However, if the pre-illuminated sample is allowed sufficient recovery in the dark, or by adding to it a secondary chemical reductant, subsequent illumination of the sample in the frozen state should then yield an EPR spectrum showing signals of  $P700^+$  as well as of reduced bound ferredoxin. These premises were experimentally confirmed and some results are shown in Fig. 2. The first signal (A) in Fig. 2 was obtained by applying five white flashes in a cylindrical flash illuminator (Ke, B., unpublished) to the EPR tube containing D-144 particles and methyl viologen and rapidly freezing the tube in liquid nitrogen 5 s after illumination. The EPR signal measured at 13 °K showed a large free-radical signal with a  $g$  value near 2 but no trace of reduced ferredoxin. Furthermore, if the EPR tube used in producing spectrum A was flash illuminated again at approximately 77 °K, no changes in the EPR spectrum were observed. Spectrum B in Fig. 2 shows the same EPR signal (A) recorded at a lower amplification to display the simple free-radical signal of  $P700^+$ . The  $g$  value of  $2.0025 \pm 0.0005$  and a linewidth of 7–8 G of the signal are consistent with that of  $P700^+$ .

When the pre-illuminated sample was allowed to stand in the dark for 10 min,

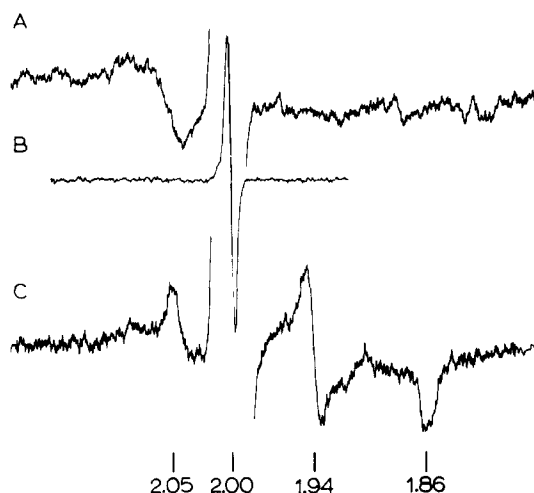


Fig. 2. (A) EPR spectrum of Photosystem-I subchloroplast particles pre-illuminated in the presence of methyl viologen. The sample composition was the same as in Fig. 1. The conditions of EPR spectroscopy were: microwave frequency, 9.18 GHz; power, 3 mW; modulation frequency, 100 KHz; modulation amplitude, 8 G; scanning rate, 200 G/min; time constant, 0.5 s; sample temperature,  $13.3 \pm 0.15$  °K. The ordinate represents  $d\chi'/dH$  in arbitrary units. (B) Same sample as in A recorded at 10  $\mu$ W of power, modulation amplitude of 4 G, 42 °K and 0.4 times the amplification; other conditions as in A. (C) Same sample as in A pre-illuminated, stored in the dark for 10 min and then frozen in the dark in liquid nitrogen. Before the EPR spectrum was recorded (conditions as for A), the sample tube was illuminated with five white flashes at 77 °K.

frozen in liquid nitrogen in the dark, and then flash illuminated at 77 °K, the EPR spectrum shown by C in Fig. 2 results. This spectrum is composed of a free-radical signal as well as a signal typical of an iron-sulfur protein, with major peaks at 2.05, 1.94 and 1.86 on a  $g$ -factor scale. The same EPR spectrum was obtained when TMPD was added to the pre-illuminated D-144 particles which contained methyl viologen and the sample frozen in dim light and then flash illuminated at 77 °K.

Considering the peculiar character of this reaction route, namely, the rapid electron discharge by  $P430^-$  and the accumulation of  $P700^+$ , and the fact that plant ferredoxins show an EPR signal only in their reduced forms, we think the evidence obtained in the reported experiments for the identity of  $P430$  with an iron-sulfur protein is significant, although admittedly indirect. Other approaches for a direct correlation between  $P430$  and the EPR signal of bound ferredoxin are being sought. A redox titration of the membrane-bound ferredoxin would not only yield basic information regarding the nature of the bound electron carrier, but may also furnish information whether the two species, one detected by optical and the other by EPR spectroscopy may be identical. Work in this area is currently in progress and results will be reported elsewhere.

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