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One-way electron discharge subsequent to the photochemical charge separation in Photosystem I★

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SUMMARY

Subsequent to the photochemical charge separation in Photosystem I, three fates are possible: (a) recombination of the photooxidized $P700^+$ and photoreduced $P430^-$; (b) a cyclic electron flow involving $P700^+$, $P430^-$ and another electron carrier present in its oxidized and reduced forms; and (c) a non-cyclic electron flow involving one electron donor reacting with $P700^+$ and another electron acceptor reacting with $P430^-$. This note deals with a fourth fate which is brought about only when an autooxidizable secondary electron acceptor is present but the secondary electron donor is either absent or blocked. In this case, only $P430^-$ reverts to the uncharged state in the dark by discharging its electron; $P700^+$ remains oxidized and reverts to the uncharged state only extremely slowly.

Recent kinetic spectroscopic investigations have uncovered a light-induced absorption change in various Photosystem-I subchloroplast particles which is consistent with the expected behavior of the primary electron acceptor of Photosystem I[1-3]. From the wavelength location, the species represented by this spectral change has been designated as "P430"[1]. The assignment of the light-induced absorption changes to the primary electron acceptor, P430, has largely been based on its onset kinetics and the corresponding decay kinetics of the coupled reactions[1-3]. Consistent with P430 and P700 being the species formed in the primary photochemical act of Photosystem I, the quantum

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Abbreviations: TMPD, *N,N,N',N'*-tetramethylphenylenediamine; DCMU, 3-(4'-dichlorophenyl)-1,1-dimethylurea.

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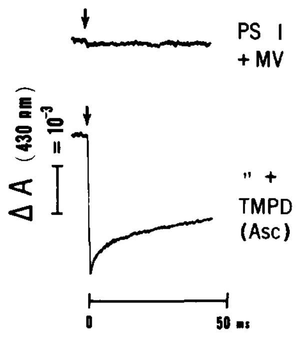
yield was found near unity[2], and an onset time of 0.1 μs or less[4]. By kinetic spectrophotometric measurements made under redox poisings, the midpoint oxidation–reduction potential of P430 was found to be −470 mV *vs* the standard hydrogen electrode[4].

Based on the assumption that the initial photochemical reaction in Photosystem I after the absorption of a photon is a charge separation, the charged species would then be represented by the oxidized $P700^+$ and reduced $P430^-$. Following the formation of these charged species, three fates are possible for their subsequent disposal, depending on the conditions and the kinds of other reactants present[2]. These are: (a) a direct recombination of $P700^+$ and $P430^-$; (b) a cyclic electron flow involving $P700^+$, $P430^-$ and a single artificial electron carrier (present in its oxidized and reduced forms) in a small reaction cycle; and (c) a non-cyclic electron flow involving one kind of electron donor reacting with $P700^+$ and another kind of electron acceptor reacting with $P430^-$. Since the spectral identification of the primary electron acceptor was made, the three possible fates of the primary reactants have been examined in detail[2] in terms of the kinetics of P700, P430 and the interacting artificial electron carrier(s), and the experimental observations have been in complete agreement with their expected behavior. It is of interest to note that all three routes result in the restoration of the primary reactants to the uncharged state so that new photochemical reactions can occur upon absorption of more light quanta.

In the course of the P430 study, we have recognized a situation that would constitute a fourth possible fate for the photochemically formed primary reactants. This reaction route occurs only if a secondary electron acceptor is present to provide a trap for the electron discharged by $P430^-$. The second provision is that the reduced form of the secondary electron acceptor reacts rapidly with oxygen and reverts back to the oxidized state. As a result of this, or in spite of this, the secondary electron carrier cannot donate electrons to $P700^+$. Otherwise, a cyclic electron flow would result. The presence of such a secondary acceptor would lead to a discharge of electrons from $P430^-$ to the secondary acceptor and then to oxygen, leaving $P700^+$ in the positively charged state. Upon sufficient illumination, all P700 are oxidized and remain so, and no further photochemical reaction of charge separation can take place upon absorption of more light quanta. This reaction route is unique in that it restores only the primary electron acceptor to the uncharged state.

The low-potential viologens fulfill the requirements mentioned above: they are efficient secondary electron acceptors for Photosystem I, the reduced semiquinone reacts rapidly with oxygen, and they do not donate electrons to $P700^+$. The flash-induced absorption change at 430 nm in a Triton-fractionated Photosystem-I subchloroplast particle in the presence of methyl viologen alone is shown by the upper trace in Fig. 1. The experimental procedures have been described previously[1–5]. The experiment was carried out immediately after the sample was pre-illuminated with two or three closely spaced red actinic flashes. Since under these conditions the $P700^+$ remained in the oxidized state, further flashing can no longer produce charge separation, and thus the



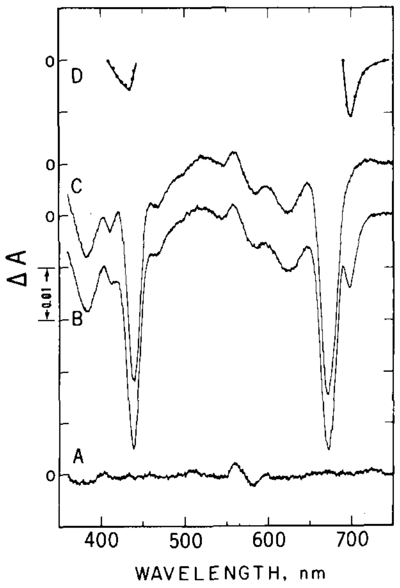


Fig. 1. Flash-induced absorption changes in Triton-fractionated subchloroplast particles (chlorophyll concn, 12 μg/ml)in the presence of methyl viologen (20 μM) alone (upper trace). Absorption change in the same reaction mixture with ascorbate (0.5 mM) and TMPD (50 μM) added (bottom trace). Experimental procedures have been described in refs 1–5. Both signals were averaged with 32 repetitive red actinic flashes (at arrow).

Fig. 2. Differenc spectra. Cuvettes containing subchloroplast particles (chlorophyll concn, 17 μg/ml) and 0.1 mM methyl viologen are placed in both the sample and reference compartments of the Cary 14 spectrophotometer. (A) Baseline (the deviation from 555 to 595 nm is inherent in this particular instrument). (B) Difference between the illuminated and unilluminated samples. Samples illuminated for 1 min by red light isolated with a Corning 2-64 filter and 2 inches of water. Incident intensity $4 \cdot 10^5$ ergs / cm^2 per s. Scan started at 5 s after cessation of illumination. Scan speed, 2.5 nm/s (156 s from 750 to 360 nm). (C) Difference spectrum taken from the same samples used in (B) which had remained in the dark compartment for an additional 5 min. (D) Difference between (B) and (C).

consequent absorption changes.

Upon additon of ascorbate and *N,N,N′,N′*-tetramethylphenylenediamine (TMPD) to reduce P700$^+$, a typical non-cyclic electron flow[1,2] represented by the biphasic-decaying signal occurred; the rapid portion is that due to P430 and the slow portion P700, and the ratio of the magnitude is about 1:4[2].

A similar one-way electron discharge in Photosystem I has previously been observed and reported by Rumberg[6] in unfractionated chloroplasts where the secondary electron donor was blocked by 3-(4′-dichlorophenyl)-1,1-dimethylurea (DCMU) at the Photosystem-II source. The author also suggested using the decreased absorption change as an assay method for establishing which substance can serve as a secondary acceptor for Photosystem I.

One consequence of the one-way electron discharge is the accumulation of P700$^+$. Since its recovery (presumably by some unknown endogenous electron donors present) is so slow, a chemical difference spectrum of P700$^+$ may be obtained from the illuminated *minus* the unilluminated sample, as shown in Fig. 2. Illumination apparently causes P700 oxidation as well as a bleaching of the bulk chlorophyll[7] as shown by the strong difference bands at 673 and 438 nm, in addition to the small band near 700 nm in Trace B, Fig. 2. The same samples were rescanned after they had remained in the dark for an additional 5 min. Now P700$^+$ had slowly reverted while the irreversibly bleached chlorophyll remained unchanged (see Trace C). The difference between (B) and (C) is typically that of P700$^+$ with absorption maxima at 701 and 435 nm. The magnitude of the 701-nm band indicates the ratio of P700 that remained oxidized to the total chlorophyll was 1:100, agreeing with that estimated separately by ferricyanide oxidation. Thus, this reaction could provide a relatively simple method for estimating the P700 content of a chloroplast sample. It might be mentioned that by using a shorter illumination time, the same P700$^+$ difference spectrum could also be produced but with much less irreversible bleaching of the bulk chlorophyll.

It is known that in P700$^+$ the ratio of the red to the blue band height is 1.45[7,8], whereas that measured in Fig. 2 is 1.95. This discrepancy is caused by the fact that 120 s after the cessation of illumination, about 25% of P700$^+$, and in 7–8 min, practically all P700$^+$, had recovered, as confirmed either by reverse scanning from 360 nm upward or by measuring the absorption decrease with time at the peak wavelengths. The recovery time is many orders slower than that due to recombination (approx. 10^{-2} s) or brought about by artificial donors.

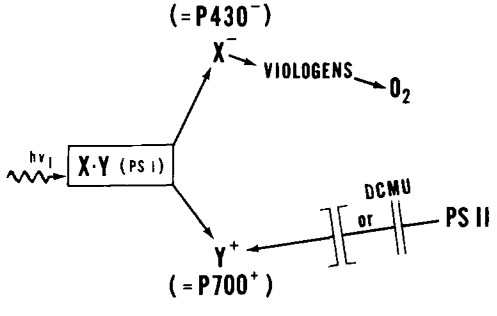


Fig. 3. Scheme for the one-way electron discharge subsequent to the photochemical charge separation in Photosystem I. An artificial secondary electron acceptor is present alone. The secondary electron donor is either absent in the subchloroplasts or blocked by DCMU in the chloroplasts.

Fig. 3 is a schematic presentation of the one-way electron discharge in Photosystem I in the presence of a secondary electron acceptor, and when the secondary donor is either absent or blocked.

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REFERENCES

1 Hiyama, T. and Ke, B. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 1010
2 Hiyama, T. and Ke, B. (1971) *Arch. Biochem. Biophys.* 147, 99
3 Hiyama, T. and Ke, B. *Proc. 2nd Int. Congr. on Photosynthesis, Stresa, Italy*, in the press
4 Ke, B. *Arch. Biochem. Biophys.*, (1972) in the press
5 Ke, B., Treharne, R.W. and McKibben, C. (1964) *Rev. Sci. Instrum.* 35, 296
6 Rumberg, B. (1964) *Z. Naturforsch.* 19B, 707
7 Ke, B., Ogawa, T., Hiyama, T. and Vernon, L.P. (1971) *Biochim. Biophys. Acta* 226, 53
8 Hiyama, T. and Ke, B. (1972) *Biochim. Biophys. Acta* 267, 160

Biochim. Biophys. Acta, 267 (1972) 595–599