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Kinetic evidence for the function of Z in isolated photosystem II reaction centers

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(D_1,D_2) Photosystem II reaction centers were studied by flash absorption spectroscopy with microsecond resolution. Without addition, absorption transients in the 660–850 nm region are due to a triplet state, probably of P-680, with a decay half-time of 700 μs (no oxygen) or 40 μs (under air). With the addition of DBMIB, presumably acting as electron acceptor, new kinetic and spectral features appear, which are attributed to P-680. A 5 μs phase of decay is present, which is pH-dependent and is attributed to donation from Z to P-680^+.

Photosynthesis; Primary reaction; Tyrosine; Photosystem II

1. INTRODUCTION

In PS II, excitation by light induces oxidation of the primary donor P-680, reducing the primary acceptor, a pheophytin I. The primary radical pair (P-680^+,I^-) subsequently evolves by electron transfer from I^- to the first quinone Q_A, and from the secondary electron donor Z to P-680^+. After a short flash, Q_A is reduced in less than 1 ns and the reduction of P-680^+ by Z lasts for periods which vary from the 50 ns domain, under physiological conditions, to that of 5–50 μs, when oxygen evolution is inhibited [1]. The reaction center of PS II has been recently isolated [2]. It contains two polypeptides, named D_1 and D_2, which hold the primary photochemical partners P-680 and I, and cytochrome *b*-559, but Q_A is absent. According to recent results obtained by site-directed mutagenesis [3] and by specific iodination experiments [4], Z is one of the tyrosines, Tyr-161, of polypeptide D_1. Thus, Z should be present in purified (D_1,D_2) PS II reaction centers, however, there is no functional evidence of its presence.

In searching for such evidence, the main limitation is based on the kinetics: since Q_A is absent, the radical pair (P-680^+,I^-) decays by recombination, with a $t_{1/2}$ of about 30 ns [5,6], much faster than donation by Z which should be in the microsecond range. It has been observed, however, that Q_A function can be reconstituted by addition of quinones [7,8]. We have found that DBMIB is capable of reconstituting Q_A function (Satoh, K. et al., in preparation), and describe its use as a tool to show that, upon addition of DBMIB, P-680^+ formed by a flash is re-reduced with kinetic behaviour attributable to Z, on the basis of previous studies with chloroplasts [9,10].

2. MATERIALS AND METHODS

(D_1,D_2) PS II reaction centers were prepared by Triton X-100 extraction of PS II membranes from spinach chloroplasts [2], with modifications which include isoelectric focusing in the

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Abbreviations: DBMIB, 2,5-dibromothymoquinone; PS, photosystem; P-680, primary electron donor in PS II; Z, tyrosine residue, secondary electron donor in PS II

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presence of digitonin (Nakane, H. and Satoh, K., submitted) and which ensures much greater stability. They were kept frozen at −70°C. For the experiments, after thawing, they were diluted in a medium comprising 50 mM Tris-HCl (pH 7.2), 0.2% digitonin and 20% by vol. of glycerol. When added, DBMIB was from a 5 mM stock solution in ethanol. Experiments at various pH values were performed with 100 mM buffers: Tris (pH 9 and 8), Hepes (pH 7) and Mes (pH 5 and 6).

For flash absorption experiments, the medium with reaction centers was in a regular 10 × 10 mm cuvette, usually maintained at 10°C. The cuvette could be roughly degassed by quick pumping with a primary vacuum pump. Absorption changes in the time range 1 μs–2 ms were recorded as in [9], except that excitation was provided by a dye laser (with broad band emission around 595 nm and energy of about 4 mJ) pumped by a 10 ns frequency-doubled Nd:YAG laser. For recording of difference spectra, matching interference filters were placed before the cuvette and in front of the detector (bandwidth, 3 nm from 660 to 700 nm; 10 nm above 700 nm).

3. RESULTS

Flash excitation of (D$_1$,D$_2$) reaction centers induces absorption changes which recover in the microsecond domain (figs 1,2). In the 660–850 nm region, the decay is practically monophasic, with a $t_{1/2}$ of 600–800 μs in degassed cuvettes or in the presence of dithionite which scavenges the oxygen (fig.1), and 40 μs under air. The difference spectrum shown in fig.3 includes a strong bleaching centered around 685 nm and a broad positive band maximum at 720 nm. In view of our previous results [6], these features are attributable to the triplet state of P-680, i.e. ^{3}P-680, which is populated as a result of charge recombination in the primary radical pair. The effect of oxygen on the decay kinetics had not been noticed earlier. It is consistent with the triplet nature of the state, and also with the absence of formation of the triplet state of β-carotene in the reaction centers [5].

Addition of DBMIB induces complex changes in the flash-induced absorption changes. Nanosecond and millisecond transients will be analyzed separately (in preparation). They are consistent with the idea that DBMIB accepts an electron from I$^-$, and then reduces cytochrome b-559, which then re-reduces P-680$^+$ in a fraction of the centers. Absorption changes attributed to transient states of β-carotene were observed around 500–550 nm and above 900 nm; they will also not be analyzed here.

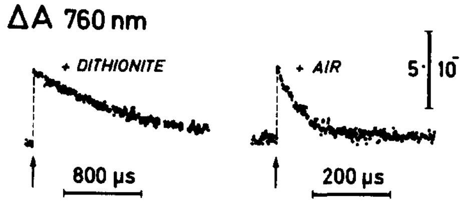

Fig.1. Absorption change at 760 nm induced by a laser flash in (D$_1$,D$_2$) PS II reaction centers (about 0.5 μM) without addition (+ air) or with addition of dithionite (0.7 mg·ml^{-1}). Average of 4 flashes; 21°C.

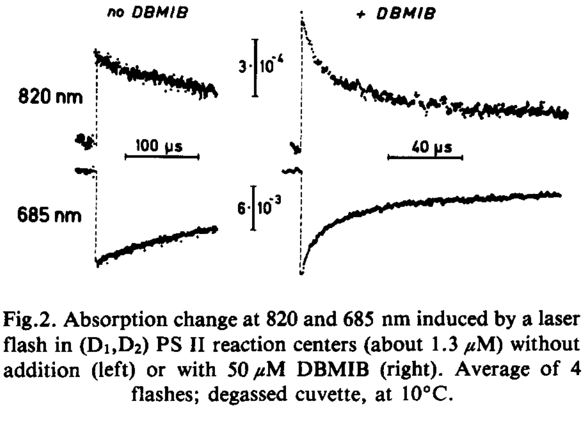

Fig.2. Absorption change at 820 and 685 nm induced by a laser flash in (D$_1$,D$_2$) PS II reaction centers (about 1.3 μM) without addition (left) or with 50 μM DBMIB (right). Average of 4 flashes; degassed cuvette, at 10°C.

The drastic kinetic changes induced by addition of DBMIB are illustrated in fig.2, at 820 and 685 nm. On our time scale, the decay is triphasic, with a fast ($t_{1/2} \approx 5\ \mu$s), an intermediate ($t_{1/2} \approx 25\ \mu$s), and a very slow phase ($t_{1/2} > 1$ ms). The decay kinetics are not influenced by oxygen concentration, a property showing that ^{3}P-680 no longer contributes to the absorption changes in the microsecond range. The difference spectrum is plotted in fig.3, in which only the part of the absorption changes which decays during 32 μs after the flash (this form of presentation was chosen in order to avoid a possible contribution by the β-carotene radical cation, which appears to be formed to a small extent and could induce slowly decaying absorption changes; in any case, this contribution would be small and the spectrum of the initial absorption changes, at the time of the flash, is not significantly different from that plotted in fig.3). The shape of the spectrum is significantly different from that of ^{3}P-680: the bleaching in the red has a different shape, and the broad band at 720 nm is replaced by a very flat absorption increase between 710 and 820 nm. The spectrum is more akin to that of a chlorophyll a cation radical, and P-680$^+$ is an obvious candidate because the

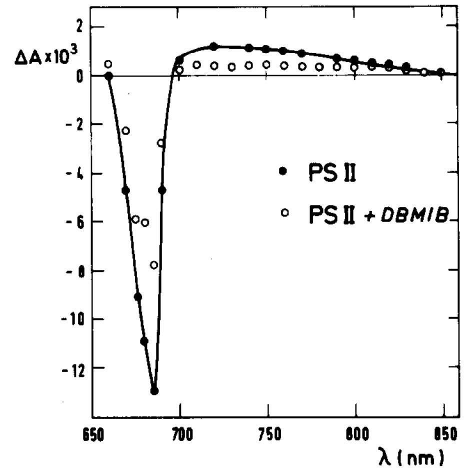

Fig.3. Difference spectra of absorption changes recorded under conditions similar to those of fig.2. (●) No DBMIB; measurement of absorption change immediately after flash. (○) Addition of 50 μM DBMIB; measurement of absorption change that decays during the first 32 μs after the flash.

main bleaching is centered around 680 nm, and also for mechanistic reasons. A difference spectrum with a bleaching including a shoulder at 670 nm has been reported recently [11] for photooxidation of P-680 in (D_1,D_2) particles.

Accepting as being correct that the absorption changes are due to P-680$^+$, the problem as to the origin of the kinetic phases of decay then arises. Measurements on a slower time scale indicate that the millisecond component is probably due to electron donation to P-680$^+$ by reduced cytochrome *b*-559. The microsecond phases (fig.4) have been studied at various pH values, in an attempt to obtain a clue to their attributions. As shown in fig.5, pH has only a small effect on the size of the initial absorption change measured at 820 nm (although the signal decrease at pH 9 is rather more evident). At the five pH values studied, the decay can be fitted by three exponential components, with approximate (pH-independent) half-times of 5 ± 1 μs, 25 ± 3 μs, and over 1 ms. The intermediate phase constitutes about 30% of the total, at all pH values, but the proportion of the fast phase varies greatly from 13% at pH 5 to 52% at pH 9 (fig.5). The pH has no effect on the decay kinetics of the absorption changes in the absence of DBMIB.

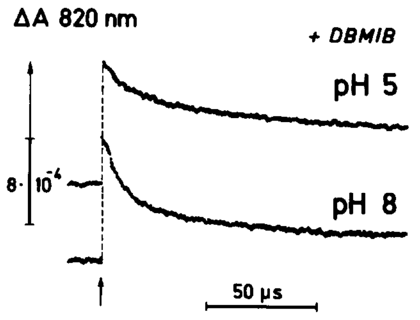

Fig.4. Kinetics of absorption change at 820 nm induced by a laser flash in (D_1,D_2) PS II reaction centers (about 2 μM), with 50 μM DBMIB, in degassed buffer at pH 5 or 8. Average of 20 experiments. 10°C.

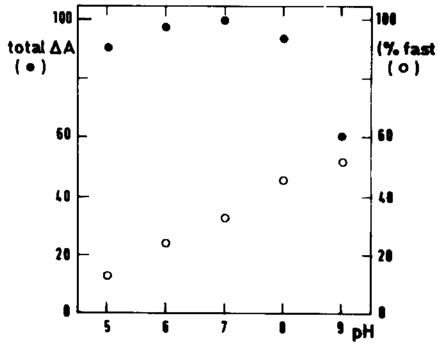

Fig.5. A plot, at five pH values, of the total absorption changes (as in fig.4) and of the fraction of signal that decays with a half-time of about 5 μs.

4. DISCUSSION

It is now well established that the (D_1,D_2) PS II reaction center is able to perform the primary step of photoinduced charge separation. Secondary reactions have also been observed, upon addition of electron acceptors like silicomolybdate [12] or plastoquinone [7,8] which are supposed to stay at the Q_A site and acceptor electrons from I^-. On the donor side, artificial donors have been shown to work [2,7,8], and intrinsic donors as well: a donor D involved in a stable triplet state [13], and a species, presumably Z, capable of undergoing photooxidation [14]. Together with other results we obtained on the effect of DBMIB (Satoh, K. et al., in preparation), the present data are best interpreted if that quinone also accepts electrons from I^-, leaving P-680 oxidized for a time much longer than the duration of primary radical pair recombination. We also obtained evidence indicating that P-680$^+$ is reduced partly by cytochrome *b*-559, in the millisecond time range.

The fast phase is similar to that previously reported in chloroplasts, after inhibition of oxygen evolution by various treatments [9,10,15], and attributed to electron donation from Z. A comparable kinetic phase has been reported in EPR experiments, and interpreted similarly [16]. An intermediate phase ($t_{1/2}$ = 22 μs), presumably identical to the 25-μs component observed here, has also been reported in Tris-treated chloroplasts but has not been fully explained [16].

Although hypothetical, our interpretation would fit with the proposal that Z is a tyrosine residue of D_1 and therefore present and potentially functional in (D_1,D_2) PS II reaction centers. The existence of slow phases of P-680$^+$ reduction may indicate that a native conformation of Z, with respect to P-680, is not retained (mostly at low pH) and perhaps also that a fraction of reaction centers are damaged. Slow reduction of P-680$^+$ may also be due to the stable oxidation of Z which would not be efficiently reduced by cytochrome *b*-559. In support of this explanation is the previous report [8] that cytochrome *b*-559 is not reducible by plastoquinol at low pH, under conditions where we find that the reduction of P-680$^+$ is predominantly slow.

REFERENCES

[1] Mathis, P. and Rutherford, A.W. (1987) in: New Comprehensive Biochemistry: Photosynthesis (Amesz, J. ed.) vol.15, pp.63–96, Elsevier, Amsterdam.
[2] Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
[3] Debus, R.J., Barry, B.A., Sithole, I., Babcock, G.T. and McIntosh, L. (1988) Biochemistry 27, 9071–9074.
[4] Takahashi, Y. and Satoh, K. (1989) Biochim. Biophys. Acta 973, 138–146.
[5] Takahashi, Y., Hansson, Ö., Mathis, P. and Satoh, K. (1987) Biochim. Biophys. Acta 893, 49–59.
[6] Danielius, R.V., Satoh, K., Van Kan, P.J.M., Plijter, J.J., Nuijs, A.M. and Van Gorkom, H.J. (1987) FEBS Lett. 213, 241–244.
[7] Chapman, D.J., Gounaris, K. and Barber, J. (1988) Biochim. Biophys. Acta 933, 423–431.
[8] Gounaris, K., Chapman, D.J. and Barber, J. (1988) FEBS Lett. 240, 143–147.
[9] Van Best, J.A. and Mathis, P. (1978) Biochim. Biophys. Acta 503, 178–185.
[10] Conjeaud, H. and Mathis, P. (1980) Biochim. Biophys. Acta 590, 353–359.
[11] Telfer, A. and Barber, J. (1989) FEBS Lett. 246, 223–228.
[12] Barber, J., Chapman, D.J. and Telfer, A. (1987) FEBS Lett. 220, 67–73.
[13] Demetriou, C., Lockett, D.J. and Nugent, J.H.A. (1988) Biochem. J. 252, 921–924.
[14] Frank, H.A., Hansson, Ö. and Mathis, P. (1989) Photosynth. Res., in press.
[15] Reinman, S., Mathis, P., Conjeaud, H. and Stewart, A. (1981) Biochim. Biophys. Acta 635, 429–433.
[16] Boska, M., Sauer, K., Buttner, W. and Babcock, G.T. (1983) Biochim. Biophys. Acta 722, 327–330.
[17] Conjeaud, H., Mathis, P. and Paillotin, G. (1979) Biochim. Biophys. Acta 546, 280–291.