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Silicomolybdate substitutes for the function of a primary electron acceptor and stabilizes charge separation in the photosystem II reaction center complex

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Effects of silicomolybdate on the charge recombination between $P680^+$ and the reduced pheophytin were studied by absorption and electron paramagnetic resonance spectroscopies in the photosystem II D1/D2/cytochrome *b*-559 reaction center complex. This preparation lacks the primary and secondary quinone acceptors, Q_A and Q_B, and exhibits the charge recombination which produces the triplet state of P680 to a large extent. In the presence of silicomolybdate, the light-induced triplet signal of P680 was almost completely eliminated at cryogenic temperatures as well as at 4°C. Under these conditions, two types of signals, one reversible and the other irreversible, which are ascribable to $P680^+$ and the cation radical of antenna chlorophyll *a*, respectively, were generated upon illumination at cryogenic temperatures. These results indicate that silicomolybdate, which is known to be an artificial electron acceptor of Q_A, rapidly receives electrons from the reduced pheophytin even at cryogenic temperatures and thus suppresses the radical pair recombination which occurs in the time range of nanosecond. $P680^+$ formed by flash excitation in the presence of silicomolybdate relaxed mainly with a long half decay time of 74 ms at 4°C. This indicates that the reduction of $P680^+$ by the secondary electron donor, Z, is significantly decreased.

Photosynthesis; Photosystem II; Radical pair recombination; Reaction center; Silicomolybdate; Triplet state

1. INTRODUCTION

Light energy absorbed by the PS II reaction center induces charge separation between the reaction center chlorophyll, P680, and the intermediate electron acceptor, pheophytin (review [1]). Electrons are transferred from the photoreduced pheophytin to the bound plastoquinone acceptor, Q_A, and then to the secondary quinone, Q_B. The photooxidized P680, on the other hand, is reduced by the electrons from water through the high potential secondary electron donor, Z, which presumably is a tyrosyl residue on the reaction center D1-protein [2–4]. Comparison of the amino acid sequences of the polypeptides of the purple bacterial reaction center with those of PS II reaction center polypeptides [5,6] as well as the isolation of the photoactive PS II RC complex [7] led us to conclude that the heterodimer of D1 and D2 proteins constitutes the PS II reaction center in a homologous manner to the L and M subunits forming the purple bacterial reaction center.

The PS II RC complex consisting of D1/D2/cytochrome *b*-559 polypeptides has been isolated from spinach [7] and pea [8], green algae, *Scenedesmus* [9] and cyanobacterium, *Synechocystis* [10]. Although these preparations retain the ability of light-induced charge separation between P680 and pheophytin [11–13], they are depleted of Q_A and Q_B plastoquinone acceptors and show high probability of charge recombination

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Abbreviations: PS II, photosystem II; EPR, electron paramagnetic resonance; DPC, 1,5-diphenylcarbazide; $P680^T$, triplet state of P680; PS II RC complex, photosystem II D1/D2/cytochrome *b*-559 reaction center complex

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which has a 30–40 ns half time [12,13]. Recently, artificial electron acceptors such as silicomolybdate or quinones were reported to be reduced in the presence of DPC under continuous illumination in the pea PS II RC complex [8,14]. However, the mechanism and the efficiency of this reaction have not been clarified.

In the present study, we have examined the effects of silicomolybdate on the recombination reaction between $P680^+$ and the reduced pheophytin in the PS II reaction center complex by absorption and EPR spectroscopies. Addition of silicomolybdate fully suppressed the charge recombination and concomitantly generated two types of chlorophyll radicals upon illumination. It is concluded that silicomolybdate efficiently accepts electrons from photoreduced pheophytin and prevents the radical pair recombination at cryogenic temperatures as well as at 4°C.

2. MATERIALS AND METHODS

The PS II RC complex consisting of D1/D2/cytochrome *b*-559 polypeptides was purified as described in [7]. This preparation contains 4–5 molecules of chlorophyll *a*, 2 molecules of pheophytin *a*, 1 molecule of *β*-carotene, but no plastoquinone 9 [7]. Flash absorption spectroscopy was performed with a split-beam spectrometer (1 *μ*s time resolution) using a laser flash (532 nm, 10 ns of half peak height duration) from the second harmonic of a Nd-YAG laser (Quanta-Ray DCR2-10) at 4°C as described in [15]. Intensity of the laser flash was not saturating. Continuous light-induced absorption spectra at low temperature were detected with a diode array apparatus as described in [16]. The EPR measurement was carried out with a Bruker EPR-200 X-band spectrometer using a continuous liquid helium flow cryostat (Oxford Instruments ESR-900) as described in [17]. The sample was illuminated with a white light from a tungsten-iodine lamp passed through a cut-off filter (R60, Toshiba) and a heat absorbing filter (HA 50, Hoya) by means of a glass fiber light guide.

3. RESULTS

3.1. *Effects of silicomolybdate on the flash-induced absorption kinetics in the PS II RC complex*

Fig.1a shows the flash-induced absorption change at 4°C in the PS II D1/D2/cytochrome *b*-559 reaction center complex (PS II RC complex). As already reported [12,13], flash excitation induces the charge separation between P680 and pheophytin. The radical pair thus formed, however, recombines rapidly with a half decay time of 30–40 ns in this complex due to lack of the quinone acceptor, Q_A [12,13]. The recombination produces $P680^T$ which decays in a microsecond time range [13]. The decay phase with a half decay time of

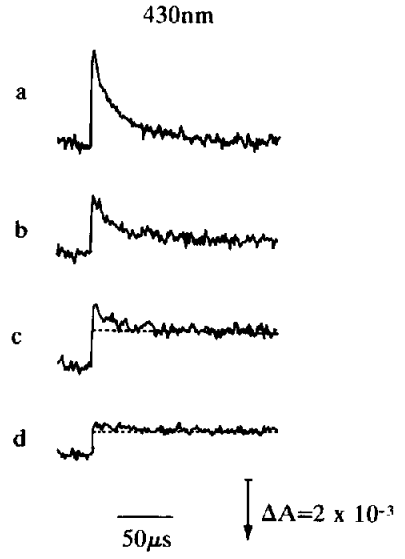


Fig.1. Effects of silicomolybdate on flash-induced absorption changes of the PS II RC complex at 4°C. (a) No addition. In the presence of (b) 0.02 mg/ml, (c) 0.1 mg/ml and (d) 0.4 mg/ml silicomolybdate. The reaction mixture contained 50 mM Tris-HCl (pH 7.2) and PS II RC complex equivalent to 5 *μ*g chl/ml. Flash was fired at 1 Hz and 256 signals were averaged.

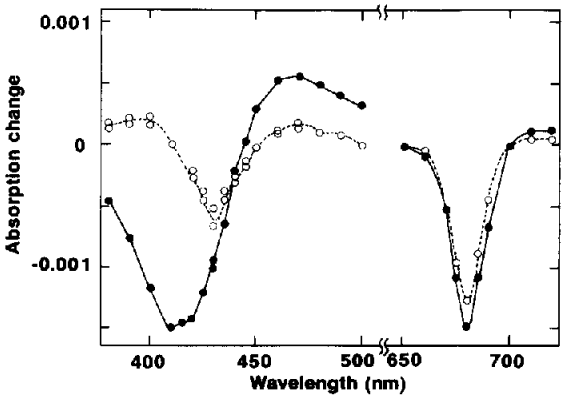


Fig.2. Difference spectra of flash-induced absorption changes of the PS II RC complex in the absence and presence of silicomolybdate. (●) No addition; (○) in the presence of 0.2 mg/ml of silicomolybdate.

20 μs in fig.1a is, thus, ascribed to the decay of $P680^T$. This is confirmed by the difference spectrum (fig.2).

Silicomolybdate is known to accept electrons directly from Q_A^- in PS II [18]. The PS II RC complex prepared from pea was recently shown to mediate electron transport from DPC to silicomolybdate under steady-state illumination [8]. This suggests that silicomolybdate accepts electrons from the reduced pheophytin. Effects of silicomolybdate on the light-induced absorption changes were analyzed using flash spectroscopy (fig.1b–d). Addition of silicomolybdate decreased the amplitude of the 20 μs decay phase of $P680^T$ and increased the slow decay phase with a half decay time of 74 ms. A small, but distinct fast 10 μs phase was detected in the presence of silicomolybdate (traces c and d). Higher concentrations of silicomolybdate partially decreased the amplitude of absorption change (fig.1d). This may correspond to the inhibitory effect of a high concentration of silicomolybdate on PS II activity as reported in the thylakoid membrane [19].

The dotted line in fig.2 also shows the difference spectrum of the absorption change of the slow decaying phase in the presence of silicomolybdate. The bandwidth of the bleaching in the blue region is narrower than that of $P680^T$ and showed blue and red bleaching peaks at 430 and 680 nm, respectively. These features of the difference spectrum suggest that the absorption changes most probably reflect that of $P680^+$. The rate of the slow decay phase was accelerated by the addition of artificial electron donors such as DPC and KI (data not shown). They are estimated to reduce $P680^+$ with apparent rate constants of $2.4 \times 10^6\ M^{-1} \cdot s^{-1}$ and $1.1 \times 10^2\ M^{-1} \cdot s^{-1}$, respectively. These results indicate that silicomolybdate efficiently suppresses the radical pair recombination and the formation of $P680^T$ by rapidly oxidizing the reduced pheophytin, and thus stabilizes $P680^+$.

3.2. *Effects of silicomolybdate on the PS II reaction at cryogenic temperatures*

Effects of silicomolybdate on the PS II reaction were also analyzed by measuring the light-induced

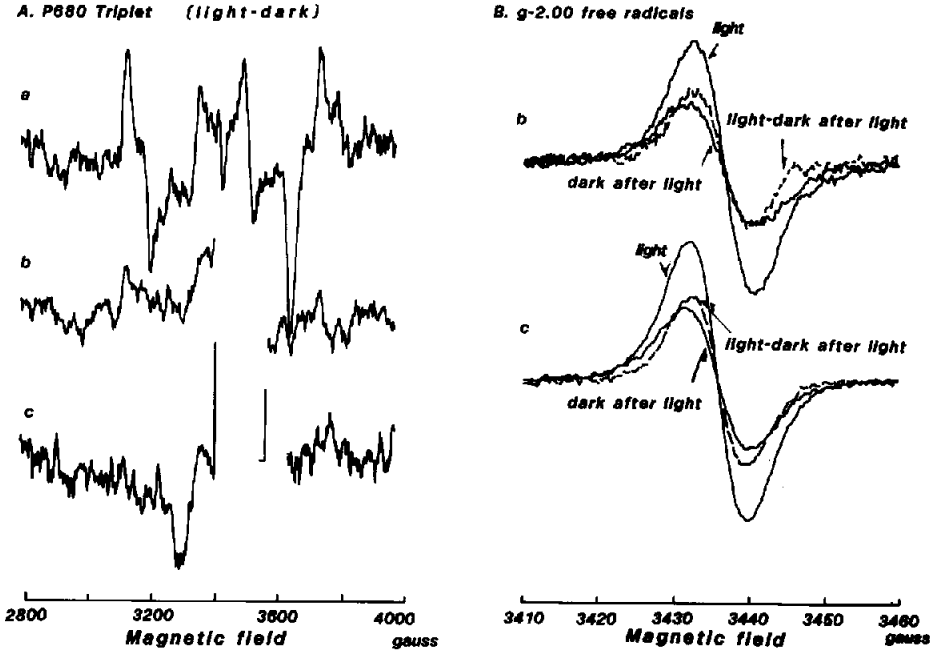


Fig.3. Effects of silicomolybdate on (A) light-induced $P680^T$ and (B) $g = 2.0$ region EPR signals in the PS II RC complex at 4 K. (a) No addition. In the presence of (b) 0.2 mg/ml and (c) 2 mg/ml silicomolybdate. Modulation amplitude, (A) 40 G and (B) 2.5 G; microwave power, 3 mW; receiver gain, 6.3×10^4. Reaction mixture contained 40 mM Tris-HCl (pH 7.2), 20% (w/v) glycerol and PS II RC complex equivalent to 100 μg chl/ml.

EPR signals at cryogenic temperatures. Illumination of the PS II RC complex at 4 K in the absence of silicomolybdate induced the spin polarized triplet signal of P680 which is produced by the radical pair recombination between $P680^+$ and the reduced pheophytin (fig.3A,a) as reported in [11]. This signal was significantly decreased and was almost undetectable in the presence of silicomolybdate at the concentration of 0.2 mg/ml and 2 mg/ml, respectively (fig.3A,b and c). The illumination in the presence of silicomolybdate generated two types of free radicals instead of the $P680^T$ signal; one is a reversible free radical with a linewidth of 6.8 G and the other is an irreversible free radical with a linewidth of 8.9 G (fig.3B,b and c). Reversible and irreversible increase of the radical at $g = 1.95$ was also detected upon illumination, which most probably arises from the reduced silicomolybdate (Mo(V)) formed by the reduction by the reduced pheophytin (data not shown). The 6.8 G narrow signal resembles that of $P680^+$ [20]. No free radical signal was detected in the dark prior to the first illumination. The 8.9 G signal was induced only upon the first illumination and was no more increased by repeated illuminations (data not shown). The accumulation of this signal during illumination was slower than that of the 6.8 G signal. In the absence of silicomolybdate, no signal was detected in the $g = 2.0$ region during as well as before and after the illumination under the same experimental conditions.

Two types of signals were also detected as light-induced optical absorption spectra in the presence of silicomolybdate at low temperatures. An irreversible bleaching (dark after light – dark) had a peak at 665 nm and a reversible bleaching (light – dark after light) at 677 nm at 245 K (fig.4). The former seems to represent the formation of $P680^+$ whereas the latter to represent the oxidation of a monomeric chlorophyll *a*. Similar absorption changes were also detected at 77 K (not shown).

Fig.5 shows the time courses of the reversible EPR signal observed in the presence of silicomolybdate at different temperatures. The relaxation of the signal was biphasic. Similar decays were observed at temperatures from 250 K to 4 K. The reversible oxidation and reduction of P680 at cryogenic temperatures suggests that silicomolybdate, or some other component which reacts with silicomolybdate, can rapidly accept electrons from the reduced pheophytin and slowly reduce $P680^+$ with a small activation energy.

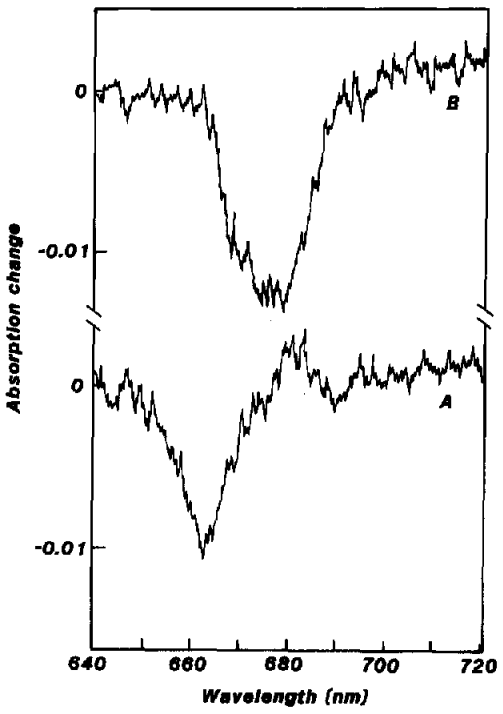


Fig.4. Difference absorption spectra of the PS II RC complex induced by continuous light in the presence of silicomolybdate at 245 K. (A) Irreversible change (dark after light – dark). (B) Reversible change (light – dark after light). Reaction mixture contained 50 mM Tris-HCl (pH 7.2), 50% (w/v) glycerol, silicomolybdate (0.2 mg/ml) and PS II RC complex equivalent to 5 μg chl/ml.

4. DISCUSSION

It is clearly demonstrated in the present study that silicomolybdate fully suppresses the formation of $P680^T$ which is produced by charge recombination between $P680^+$ and the reduced pheophytin at cryogenic as well as at higher temperatures. Since the suppression concomitantly induced stabilization of $P680^+$, it is concluded that silicomolybdate inhibits the radical pair recombination. This cannot be explained by the direct quenching of $P680^T$. These results extend the recent report by Nugent et al. [21] who showed that silicomolybdate partially suppresses $P680^T$ formation in a PS II RC complex from pea by EPR measurement under continuous illumination. Silicomolybdate, whose molecular

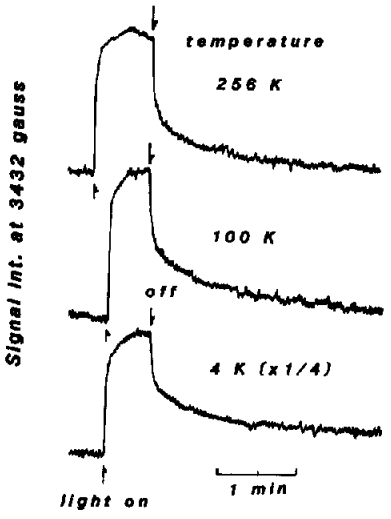
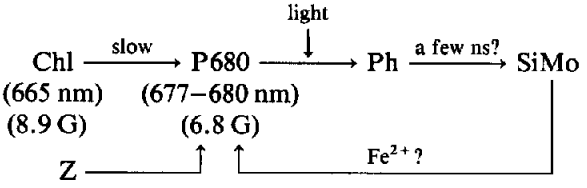


Fig.5. Time courses of the light-induced $g = 2.0$ free radical signal in the PS II RC complex in the presence of silicomolybdate at different temperatures. Experimental conditions were the same as those in fig.3, except that time constant and modulation amplitude were 160 ms and 2.5 G, respectively. Microwave power was 10 mW and receiver gain was 6.3×10^4. Reversible portion of the signal at 3432 G was shown mainly.

structure is totally different from plastoquinone, replaces the function of Q_A, and can efficiently accept electrons from the reduced pheophytin.

In the presence of silicomolybdate, two types of chlorophyll *a* free radicals were induced upon illumination. One is reversibly induced even at 4 K. This component gives an EPR signal with a narrow 6.8 G band and has a bleaching peak at 677 nm. This signal, therefore, is most probably ascribable to $P680^+$. A similar reversible optical spectrum, peaking at 680 nm but with a shoulder at 672 nm, was reported in the pea PS II RC complex under continuous illumination in the presence of silicomolybdate [22]. The other component is irreversibly induced by the first illumination at low temperatures. This component gives rise to an EPR signal with a broad 8.9 G width and has a bleaching peak at 665 nm. Therefore, this signal seems to originate from a monomeric chlorophyll *a* molecule, which is situated nearby P680 and is oxidized by $P680^+$, rather than $P680^+$ itself. The shape and characteristics of the 8.9 G irreversible EPR signal seems to be similar to that previously assigned to be $P680^+$ detected by EPR measurement in the pea PS II RC complex in the presence of ferricyanide [23]. The $g = 2.0024$, 10 G width free radical has been reported in PS II particles [24,25] or in thylakoid membranes [26] and was ascribed to the cation radical of chlorophyll *a* [26] that donates electrons to $P680^+$ at cryogenic temperature in competition with reduced cytochrome *b*-559 [25]. Mixing of a small amount of the 6.8 G $P680^+$ signal with this 10 G signal may explain the 8.9 G signal observed in this study, although further work seems to be required before a conclusion is made.

Formation of $P680^+$ by a flash excitation in the presence of silicomolybdate indicates that this component efficiently accepts electrons from the reduced pheophytin at a rate faster than that of the charge recombination reaction between $P680^+$ and the reduced pheophytin. Non-heme iron (Q_{400}), which interacts with Q_A plastoquinone in normal PS II and seems to remain partially in the RC complex [23], may intermediate electron transfer reaction between the reduced pheophytin and silicomolybdate. However, turnover of this component has not been detected yet [23]. Pathway of electrons in the PS II RC complex may be formulated as below by summarizing the results,



where Chl, Ph and SiMo represent chlorophyll *a*, pheophytin *a* and silicomolybdate, respectively.

The secondary electron donor, Z, is expected to be present in the PS II RC complex since this component is presumably a tyrosyl residue on the D1-protein [2–4,27]. Z is assumed to exist near the inner surface of the thylakoid membrane in the vicinity of P680 [28]. When oxygen evolving activity was inhibited in the thylakoid membranes or PS II particles, Z is known to donate electrons to $P680^+$ with a half-decay time of 5–7 μs at pH 7 [29]. This rate is 10^4 times faster than the decay rate of the major slow phase (74 ms) observed in the PS II RC complex in the presence of silicomolybdate (fig.1c and d). The slow dark reduction of $P680^+$ with a half-decay time of 7 ms has been reported in the PS II preparation (CP2-b) isolated

from a thermophilic cyanobacterium, *Synechococcus* sp. This preparation retains Q_A, but lacks functional Z [30]. The major slow reduction phase of $P680^+$ observed in the present work may thus reflect the modification of protein tertiary structure on the oxidizing side by detergent and/or silicomolybdate. A small EPR signal of Z^+ (5% of total reaction center) was recently reported to be photo-accumulated in the pea PS II complex in the presence of silicomolybdate [21]. Partial turnover of Z was also suggested in the spinach PS II RC complex in the presence of 2,5-dibromothymoquinone (DBMIB) from the observation that a part of $P680^+$ decayed with a half-decay time of 5 μs [31]. The rapid 10 μs decay phase of $P680^+$ observed in the present study in the presence of silicomolybdate (fig.1c and d), therefore, may reflect the reduction of $P680^+$ by Z in 30–40% of the PS II RC complex.

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REFERENCES

[1] Satoh, K. (1985) Photochem. Photobiol. 42, 845–853.
[2] Takahashi, Y., Takahashi, M. and Satoh, K. (1986) FEBS Lett. 208, 347–351.
[3] Ikeuchi, M. and Inoue, Y. (1987) FEBS Lett. 210, 71–76.
[4] Debus, R.J., Barry, B.A., Sithole, I., Babcock, G.T. and McIntosh, L. (1988) Biochemistry 27, 9071–9074.
[5] Trebst, A. (1986) Z. Naturforsch. 41c, 240–245.
[6] Michel, H. and Deisenhofer, J. (1986) in: Encyclopedia of Plant Physiology 19, Photosynthesis III (Staehelin, L.A and Arntzen, C.J. eds) pp. 371–381, Springer-Verlag, Berlin.
[7] Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
[8] Barber, J., Chapman, D.J. and Telfer, A. (1987) FEBS Lett. 220, 67–73.
[9] Demetriou, C., Lockett, C.J. and Nugent, J.H.A. (1988) Biochem. J. 252, 921–924.
[10] Gounaris, K., Chapman, D.J. and Barber, J. (1989) Biochim. Biophys. Acta 973, 296–301.
[11] Okamura, M.Y., Satoh, K., Isaacson, R.A. and Feher, G. (1987) in: Progress in Photosynthesis Research, vol. 1 (Biggins, J. ed.) pp. 379–381, Martinus Nijhoff, Dordrecht.
[12] 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.
[13] Takahashi, Y., Hansson, Ö., Mathis, P. and Satoh, K. (1987) Biochim. Biophys. Acta 893, 49–59.
[14] Gounaris, K., Chapman, D.J. and Barber, J. (1988) FEBS Lett. 240, 143–147.
[15] Itoh, S. and Iwaki, M. (1989) FEBS Lett. 243, 47–52.
[16] Itoh, S. and Iwaki, M. (1988) Biochim. Biophys. Acta 943, 32–38.
[17] Itoh, S., Iwaki, M. and Ikegami, I. (1987) Biochim. Biophys. Acta 893, 508–516.
[18] Giaquinta, R.T. and Dilley, R.A. (1975) Biochim. Biophys. Acta 387, 288–305.
[19] Hirano, M., Satoh, K. and Katoh, S. (1981) Biochim. Biophys. Acta 635, 476–487.
[20] Malkin, R. and Bearden, A.J. (1975) Biochim. Biophys. Acta 396, 250–259.
[21] Nugent, J.H.A., Telfer, A., Demetriou, C. and Barber, J. FEBS Lett., in press.
[22] Telfer, A. and Barber, J. (1989) FEBS Lett. 246, 223–228.
[23] Telfer, A., Barber, J. and Evans, M.C.W. (1988) FEBS Lett. 232, 209–213.
[24] De Paula, J.C., Innes, J.B. and Brudvig, G.W. (1985) Biochemistry 24, 5114–5120.
[25] Thompson, L.K. and Brudvig, G.W. (1988) Biochemistry 27, 6653–6658.
[26] Visser, J.W.M. and Rijgersberg, C.P. (1975) in: Proceedings of the 3rd International Congress on Photosynthesis (Avron, M. ed.) pp. 399–408, Elsevier, Amsterdam.
[27] Takahashi, Y. and Styring, S. (1987) FEBS Lett. 223, 371–375.
[28] Itoh, S., Isogai, Y., Tang, X.-S. and Satoh, K. (1987) in: Progress in Photosynthesis Research, vol. 1 (Biggins, J. ed.) pp. 483–486, Martinus Nijhoff, Dordrecht.
[29] Conjeaud, H., Mathis, P. and Paillotin, G. (1979) Biochim. Biophys. Acta 546, 280–291.
[30] Takahashi, Y. and Katoh, S. (1986) Biochim. Biophys. Acta 848, 183–192.
[31] Mathis, P., Satoh, K. and Hansson, Ö. FEBS Lett., in press.