Biochimica et Biophysica Acta 932 (1988) 91–96
Elsevier

BBA 42694

Yield and lifetime of the primary radical pair in preparations of Photosystem II with different antenna size

Örjan Hansson, Jacques Duranton and Paul Mathis

Service Biophysique, Département de Biologie, CEN Saclay, Gif sur Yvette (France)

(Received 29 June 1987)

Key words: Photosynthesis; Photosystem II; Electron transfer; Radical pair; Flash absorption spectroscopy

Several types of photosynthetic membrane fraction enriched in the Photosystem II reaction center were studied by flash absorption at 820 nm, with nanosecond resolution, to determine the lifetime of the primary radical pair and the extent of its formation when secondary electron transfer is inhibited. It results that the biradical lifetime varies largely, with halftimes from 3 to 32 ns, the shorter values being obtained with the larger, presumably more intact fractions. The amount of biradical varies from 28% to 47% (or from 55% to 67% according to the interpretation of the data).

Introduction

The reaction centers of purple bacteria and of PS II in plants show many similarities, both structural and functional. Flash excitation of bacterial reaction centers produces the radical pair P^+I^- (P, primary electron donor; I, primary acceptor, bacteriopheophytin) which, under conditions where the forward electron transfer has been blocked, recombines with $t_{1/2} \approx 10$ ns [1]. In PS II, it was proposed that a similar charge recombination of the radical pair P^+I^- (where P is P-680; I is pheophytin *a*) is the source of the variable chlorophyll fluorescence (lifetime, 2–4 ns) that develops upon reducing the plastoquinone acceptor, Q_A [2–4]. Recently, we reported on flash-absorption studies in a PS II reaction center complex (the D1/D2/cytochrome *b*-559 complex [5]) with about 5 Chl/P-680. We found a significantly longer halftime of 32 ns for the P^+I^- decay measured at 820 nm [6], as also reported by others [7]. The recombination resulted in the formation of the triplet state of P-680 with a temperature-dependent quantum yield (23% at 276 K; 80% at 10 K). The primary quinone, Q_A, is absent in this preparation, and thus the recombination reaction could be observed without addition of a reductant. A PS II core complex preparation with about 60 Chl/P-680 [8] also showed a P^+I^- biradical state lasting 20–25 ns, but with a nanosecond time resolution this state could be observed only after chemical reduction of Q_A [6].

The halftimes that we have found for the P^+I^- recombination in PS II are significantly longer than the 1–6 ns reported by others [2,9–11]. A possible cause for the discrepancy could be variations in antenna size between different preparations. With a simple kinetic model [12,13], assuming that the biradical is in thermodynamic equi-

Abbreviations: Chl, chlorophyll; ΔA, absorption change; I, primary electron acceptor; P, P-680: primary electron donor; PS II, Photosystem II; Q_A, primary quinonic acceptor; Z, secondary electron donor.

Correspondence: Ö. Hansson, (present address:) Department of Biochemistry and Biophysics, Chalmers Institute of Technology and University of Göteborg, S-41296 Göteborg, Sweden.

librium with the chlorophyll singlet excited state, one would expect a decrease in the P^+I^- lifetime when the antenna size increases. In the nanosecond time domain, the pseudo-steady-state concentration of the state P^+I^- should decrease in parallel with the excitation residing preferentially in the antenna. Here we report on flash-absorption spectroscopic studies of the P^+I^- radical pair in six different PS II preparations. We find large variations in the P^+I^- lifetime, decreasing with increasing antenna size. However, the yield of P^+I^- relative to $P^+Q_A^-$ formed under oxidizing conditions was found to be much more constant.

Materials and Methods

The PS II reaction center (the D1/D2/cytochrome *b*-559 complex) was prepared from spinach as described in Ref. 5. It contained about 4 Chl *a*/cytochrome *b*-559, as determined by optical absorption spectroscopy. Pigment extraction and HPLC purification yielded a Chl *a*/pheophytin ratio of about 3–4 and a Chl *a*/β-carotene ratio of 4. Following the procedure of Nanba and Satoh [5], we found about 15 Chl *a*/pheophytin anion accumulated with continuous illumination in the presence of dithionite and 2 μM methylviologen. In the absence of methylviologen this ratio decreased to 10. Oxygen-evolving PS II-enriched membranes were prepared according to Ref. 14. After a Tris treatment (0.8 M Tris (pH 8.4) at about 1 mg Chl/ml) the membranes were centrifuged and then resuspended at 0.35 mM chlorophyll in a buffer comprising 50 mM phosphate (pH 6.9)/50 mM NaCl/300 mM sucrose. Oxygen-evolving and non-oxygen-evolving PS II core complexes from spinach with about 60 Chl/PS II, prepared as in Refs. 15 and 16, respectively, were the kind gifts of Drs L.G. Franzén and Y. Takahashi, respectively. Non-oxygen-evolving PS II core complex and PS-II-enriched membranes from *Chlamydomonas reinhardtii* mutant F54-14, with about 50 and 115 Chl/PS II, respectively (Ref. 17 and De Vitry, C., personal communication), were kindly provided by Dr. C. de Vitry.

Flash-induced absorption changes at 820 nm were measured with an apparatus described previously [6]. The excitation pulse was from a frequency-doubled mode-locked Nd-YAG laser (532 nm; duration about 20 ps; 0.2 Hz repetition rate; intensity of ≈ 3 mJ · cm^{-2} before attenuation). The measuring light from a laser diode (Telefunken, type TXSK, 820 nm) was focussed through the cuvette on a silicon photodiode (Lasermetrics, model 3117), the output of which was amplified 100 × in two stages (Nuclétude, 1 GHz) and then fed through 50 Ω into a transient digitizer (Tektronix, type R7912 with a 7A19 amplifier). The response time of the apparatus was 1.0 ns. The sample cuvette was cooled at +3°C. Optical paths are given in the figure legends.

Results

Flash excitation of PS II reaction centers induces an immediate absorption increase at 820 nm which decays monoexponentially to an apparent constant level (Fig. 1a). The halftime of the decaying component varies between 25 and 32 ns depending on preparation and was ascribed by us to the decay of the primary radical pair P^+I^- [6]. The signal remaining at the end of the 200 ns time window (Fig. 1a) was ascribed to the triplet state of P-680, which was found to decay with $t_{1/2} = 30$ μs at 276 K [6]. Addition of ferricyanide (0.5 mM) or dithionite (1 mM) to PS II reaction centers does not influence the nanosecond absorption transient (not shown).

In contrast, the signals obtained from other PS II preparations depend on redox conditions and also on the intactness of the PS II electron donor side (Figs. 1b, c; 2 and 3). Under oxidizing conditions, PS II preparations devoid of the oxygen-evolving system but with intrinsic plastoquinone electron acceptors show a flash-induced absorption increase at 820 nm that decays very slowly on the time-scale used (Figs. 1b; 2a, c; 3c). This is attributable to the formation of the $P^+Q_A^-$ state which subsequently decays in the microsecond time range due to electron donation from Z to P^+ [18]. With an oxygen-evolving PS II core preparation, the ΔA shows multiphasic decay kinetics (Fig. 3a) as observed previously [19,20] and attributed to the S-state-dependent kinetics of electron transfer from Z to P^+ [21]. Using an absorption coefficient of 7000 $M^{-1} \cdot cm^{-1}$ for P^+ [22], the stoichiometry of chlorophyll to P^+ can be calcu-

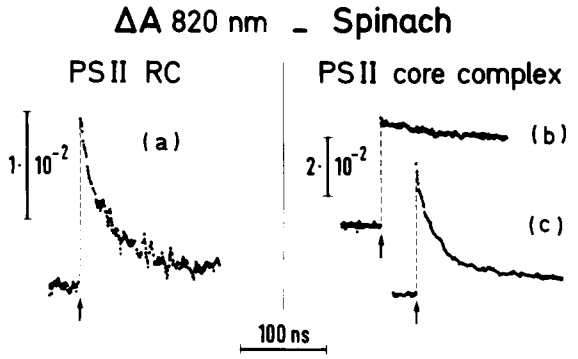

Fig. 1. Flash-induced absorption changes at 820 nm in PS II reaction centers ((a) 23 μM chlorophyll and core complexes (b, c) 450 μM Chl *a* from spinach). Optical paths in the cuvette: (a) 13 mm (measurement) and 5 mm (excitation); (b, c) 10 mm (measurement) and 1 mm (excitation). (a) No addition; (b) addition of 80 μM potassium ferricyanide; (c) further addition of 12 mM dithionite. Average effect of 10 (a, c) or 5 (b) flashes.

lated from the initial ΔA in the above experiments. The numbers obtained are somewhat larger than the ones obtained by other methods (Table I). The discrepancies are attributed to incomplete saturation of the signals with the 20 ps laser flash used in this work.

Two properties of our excitation pulse make it non-ideal for saturation studies: its short duration, which tends to favor conditions of singlet-singlet annihilation, and its wavelength (532 nm), which falls in a region of low absorption where presumably carotenoids are the major absorbing species. It is not clear that they always transfer excitation energy maximally to the reaction centers. For reasons that are not understood, PS II core complex preparations with reported similar chlorophyll to reaction center ratios show different saturation behavior. A correct saturation curve was obtained with the digitonin core complex, but for the other two core complex preparations only a qualitative estimate of the degree of saturation was obtained by attenuating the laser flash energy to 50% with a neutral density filter. This resulted in a decrease in the signals to 83% (*C. reinhardtii* core complex) and 66% (oxygen-evolving core complex from spinach) of those obtained with the maximum available laser flash energy. Also, PS-II-enriched membrane preparations with larger antenna sizes are still difficult to saturate completely with our picosecond laser pulse.

Addition of dithionite to PS II core complex or enriched membrane preparations slightly increases the size of the absorption transients, and the decay rates are markedly increased (Figs. 1c; 2b, d; 3b, d). Assuming that, under these conditions, the ΔA is due to the formation and subsequent recombination of the primary radical pair P^+I^- (see the Discussion about this assumption), we obtain, with dithionite, about 0.65 P^+I^- per $P^+Q_A^-$ formed under oxidizing conditions in the PS II core complex preparations. This ratio is only slightly reduced in the PS-II-enriched membrane preparations (Table I). For these calculations an absorption coefficient of 5400 $M^{-1} \cdot cm^{-1}$ was used for the pheophytin *a* anion (I^-) at 820 nm [23].

The decay kinetics of the absorption transients obtained in the presence of dithionite vary between different PS II preparations (Table I). The digitonin PS II core complex in Fig. 1c shows decay kinetics largely similar to those of the PS II reaction center (Fig. 1a). The other two PS II core complex preparations show significantly shorter halftimes (Figs. 2b, 3b, Table I). A biexponential

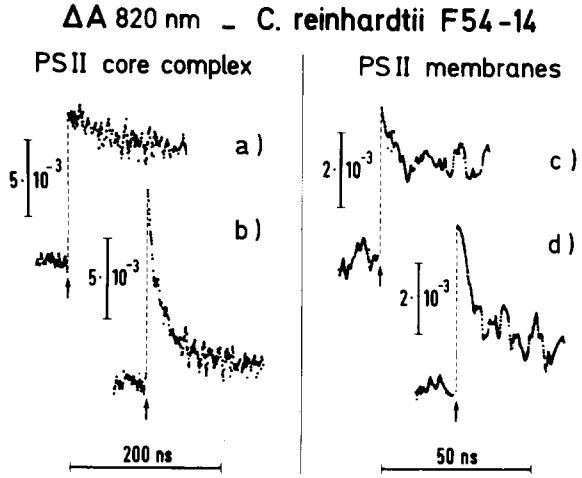

Fig. 2. Flash-induced absorption changes at 820 nm in PS II core complexes (left, 120 μM chlorophyll) and PS-II-enriched membranes (right, 90 μM chlorophyll) from *C. reinhardtii* mutant F54-14. Optical paths in the cuvette: 10 mm (measurement) and 4 mm (excitation). Addition of 55 μM potassium ferricyanide and 0.22 mM phenyl-*p*-benzoquinone. (b, d) Further addition of 1.3 mM dithionite. Average effect of 20 (a, b), 40 (c) or 80 (d) flashes.

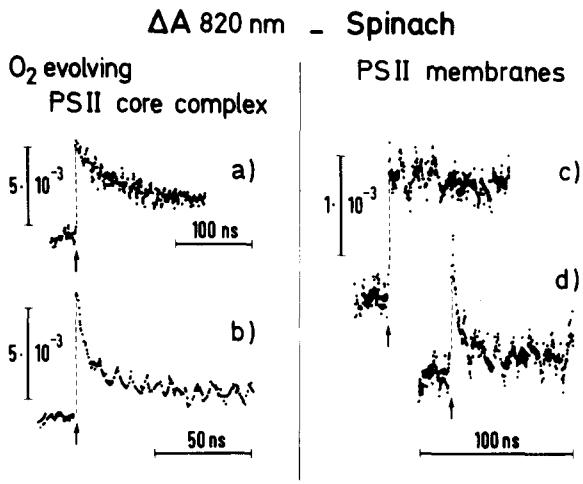

Fig. 3. Flash-induced absorption changes at 820 nm in O_2-evolving PS II core complexes (left, 130 μM chlorophyll) and Tris-washed PS-II-enriched membranes (right, 62 μM chlorophyll) from spinach. Optical paths in the cuvette: left, 10 mm (measurement) and 4 mm (excitation); right, 13 mm (measurement) and 5 mm (excitation). Additions: (a) 0.2 mM 2,5-dichloro-*p*-benzoquinone; (b) further addition of 1.6 mM dithionite; (c) 1.4 mM potassium ferricyanide; (d) 0.26 mM dithionite. Average effect of 20 (a), 80 (b) or 320 (c, d) flashes.

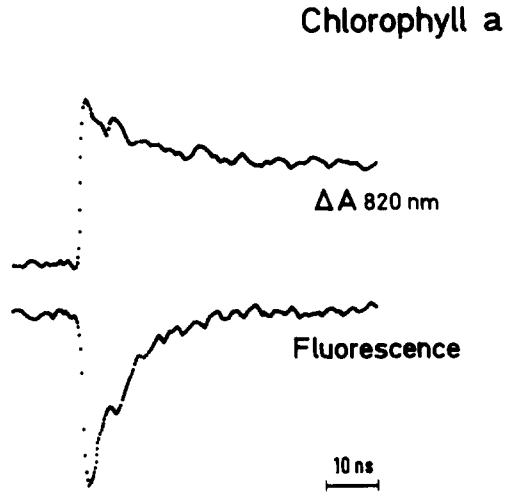

Fig. 4. Absorption change and fluorescence decay at 820 nm measured with Chl *a* (130 μM) in ethanol/glycerol (1:1). Optical paths in the cuvette: 10 mm (measurement) and 4 mm (excitation). The total ΔA at 820 nm is 0.012. The signals show the presence of a second laser pulse, of small amplitude, at 7 ns after the first.

decay with halftimes of 2 ns (50%) and 10 ns (50%) was found for the oxygen-evolving PS II core complex in Fig. 3b. The PS-II-enriched membrane preparations with large chlorophyll/reaction center ratios show monoexponential decays with halftimes of 4 (*C. reinhardtii*) and 3 ns (spinach), respectively (Figs. 2d, 3d, Table I). In the latter case, the signal size is partially reduced and the decay halftime somewhat overestimated owing to the apparatus response time. The overestimation is certainly less than 1 ns and the halftime for spinach membranes should be approx. 2.5 ns.

Flash absorption experiments were also per-

TABLE I

SUMMARY OF FLASH-INDUCED ABSORPTION DATA OBTAINED FROM DIFFERENT PHOTOSYSTEM II PREPARATIONS AT 820 nm

Chlorophyll P^+ is the ratio of chlorophyll to oxidized P-680 obtained under oxidizing conditions (see figure legends) with the maximum available laser energy. Decay halftimes were measured under reducing conditions. P^+I^-/P^+ ratio obtained from the ΔA measured under reducing or oxidizing conditions, with the assumption that, under reducing conditions, the ΔA is due to P^+ and I^- only (column a) or to P^+I^- in a fraction of the reaction centers and to excited chlorophyll in the remainder (column b). The ratios given are averages of 2–5 different experiments. Molar absorption coefficients: 7000 (P^+); 5400 (I^-); 4700 (excited chlorophyll).

Material	Chlorophyll/PS II (published)	Chlorophyll/P^+ (this work)	decay $t_{1/2}$ (ns)	P^+I^-/P^+	
				a	b
Reaction center	5 (5)	–	25–32	–	–
Spinach core complex	60 (8)	78	4(15%), 25(85%)	0.62	0.39
C. reinhardtii core complex	50 (17 [a])	68	14	0.67	0.47
Oxygen-evolving core complex	66 (15)	102	2(50%), 10(50%)	0.64	0.42
C. reinhardtii PS II membranes	115 [a]	150	4	0.60	0.36
Spinach PS II membranes	200 (30)	287	3	0.55	0.28

[a] De Vitry, C., personal communication.

formed with pure Chl *a* in solution in order to determine the absorption coefficient at 820 nm of the Chl *a* singlet excited state. As shown in Fig. 4 (upper trace), an instantaneous absorption increase is followed by a biphasic decay with a fast phase ($t_{1/2} = 5.5$ ns) and a slow phase, the decay of which was not measured precisely. The Chl *a* fluorescence was also measured at 820 nm under the same experimental conditions; it decays with a halftime of 4.8 ns (Fig. 4, lower trace). The fast phase of ΔA is attributed to the decay of the singlet excited state, in agreement with the fluorescence curve, and the slow decay to the decay of the triplet state. The slow phase represents 51% of the total initial ΔA. Knowing approximately the yield of Chl *a* triplet state from the singlet excited state (about 64% [24]), and assuming an absorption coefficient of about 3800 $M^{-1} \cdot cm^{-1}$ for the triplet state at 820 nm [22], it is then possible to evaluate that the singlet state absorption coefficient at 820 nm is 4800 $M^{-1} \cdot cm^{-1}$. This agrees with the value of 4700 $M^{-1} \cdot cm^{-1}$ which can be deduced from the data of Nuijs et al. (Fig. 1 in Ref. 10).

Discussion

The flash-absorption data that we obtain here under oxidizing conditions, with thylakoid fractions essentially devoid of Photosystem I, fit quite well those that can be expected for P-680$^+$ in PS II (a minor rapid decay observed with the *C. reinhardtii* core complex is not expected, however). Under reducing conditions, therefore, ΔA can also be reasonably attributed to the PS II reaction center. The primary biradical P^+I^- is an obvious candidate to account for the ΔA, as we could show before with the (D1/D2/cytochrome *b*-559) reaction center [6]. However, it has been proposed that an equilibrium, Chl* *a* $\leftrightharpoons$ P-680* $\leftrightharpoons$ P^+I^-, is rapidly established upon excitation of the reaction centers with Q_A reduced [12,13,25] (Chl* *a* and P-680* represent the singlet excited state of antenna Chl *a* and of P-680). We therefore have to consider that hypothesis also and the possibility that the ΔA is due in part to the biradical P^+I^- and in part to Chl* *a* or P-680* (these two species have not been distinguished in our ΔA calculations).

If the reported ΔA, under reducing conditions, is due essentially to P^+I^-, it follows that this biradical is formed in only about 60% of the reaction centers (Table I, column a), with a rather small variation from one material to the other, except for isolated reaction centers in which we found a biradical in about one-third of the centers [6]. We have no detailed explanation for the incomplete charge separation, but it may be that, after the initial process of energy transfer (when Q_A is prereduced), P-680* has a 60% probability of decaying into charge separation and a 40% probability of going via other routes, which could explain the variable fluorescence and the center-to-center energy transfer, but would not be directly detectable with our technique. On the other hand, the ΔA we observed could be due to both P^+I^- and excited states. If we assume that the total concentration of P^+I^- and of excited states is equal to the concentration of P^+ that we obtain when Q_A is oxidized, then the fraction of reaction centers that are in the state P^+I^- can be calculated (column b in Table I, absorption coefficients as indicated in the table legend). It appears that, when Q_A is reduced, the fraction of reaction centers in the state P^+I^- is about 47% in the smallest PS II core complex and that this fraction decreases with antenna size to about 28% in the largest PS-II-enriched membranes. This result tends to support the hypothesis considered. It also appears that antenna size is not the only factor in the equilibrium, since preparations with similar antenna size give differing results.

The experiments reported here were done under conditions of rather high picosecond excitation energy (about five photons per reaction center and per pulse for the core complex preparations). We suppose that eventual multi-excitation phenomena have decayed in less than our time resolution and that at most only one excitation remains per reaction center in our measurements.

Whereas the ΔA/reaction center varies little within our series of PS II materials, the decay kinetics span a range of at least one order of magnitude and in two cases display a biphasic behavior. As a general trend, the kinetics are faster in the bigger, more intact particles such as the spinach or *C. reinhardtii* PS II membranes. The spinach oxygen-evolving core complex, pre-

sumably more intact than the same core complex prepared with digitonin, also gives faster kinetics (Table I). This general trend is in favor of an equilibrium between P^+I^- and excited states, which provide additional routes for deexcitation. However, other factors could play a dominant role, such as the electrical charge on Q_A^- [1], the eventual presence of an Fe^{2+} ion interacting with Q_A^- [1] or the more or less preserved connection between the reaction center and the PS II antenna in response to detergent action.

A comparison between the absorption transient and the fluorescence decay would certainly be very fruitful. This comparison has revealed interesting discrepancies in the case of bacterial reaction centers [1,26–28]. Fluorescence decays have usually been measured in intact chloroplasts or algae, in which our measurements are not possible. The slower fluorescence component usually has a lifetime of about 2 ns ($t_{1/2} \approx 1.4$ ns) [2–4,29], which is significantly shorter than that found by us in PS II membranes ($t_{1/2} \simeq 3$ ns). A 4 ns lifetime ($t_{1/2} \approx 2.8$ ns) has been reported for both fluorescence and absorption transients in small PS II particles prepared by a rather harsh treatment with Triton X-100 [9]. These absorption transients are certainly faster than those we could predict on the basis of our own data. Fast kinetics were also reported by Holzwarth et al. [11] for a rather intact PS II particle, the recombination of the P^+I^- state being assigned a halftime of about 1 ns. These data seem to be perfectly sound. The discrepancy with our own data may originate in the different biological materials or in the different flashing conditions.

Acknowledgements

We would like to thank Drs. C. de Vitry, L.-G. Franzén and Y. Takahashi for kindly providing some PS II preparations for this study, Dr. K. Brettel for his contribution in setting up the nanosecond apparatus and Drs. A.W. Rutherford and S. Styring for stimulating discussions. Ö.H. is recipient of a post-doctoral fellowship from the Swedish Natural Science Research Council.

References

1 Schenck, C.C., Blankenship, R.E. and Parson, W.W. (1982) Biochim. Biophys. Acta 680, 44–59.
2 Klimov, V.V. and Krasnovskii, A.A. (1981) Photosynthetica 15, 592–609.
3 Karukstis, K.K. and Sauer, K. (1983) J. Cell. Biochem. 23, 131–158.
4 Holzwarth, A.R. (1986) Photochem. Photobiol. 43, 707–725.
5 Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
6 Takahashi, Y., Hansson, Ö., Mathis, P. and Satoh, K. (1987) Biochim. Biophys. Acta 893, 49–59.
7 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.
8 Satoh, K. and Mathis, P. (1981) Photobiochem. Photobiophys. 2, 189–198.
9 Shuvalov, V.A., Klimov, V.V., Dolan, E., Parson, W.W. and Ke, B. (1980) FEBS Lett. 118, 279–282.
10 Nuijs, A.M., Van Gorkom, H.J., Plijter, J.J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 848, 167–175.
11 Holzwarth, A.R., Brock, H. and Schatz, G.H. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. 1, pp. 61–65, Martinus Nijhoff, Dordrecht.
12 Schatz, G.H. and Holzwarth, A.R. (1986) Photosynth. Res. 10, 309–318.
13 Mathis, P. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. 1, pp. 151–160, Martinus Nijhoff, Dordrecht.
14 Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 23, 533–539.
15 Franzén, L.-G., Styring, S., Etienne, A.-L., Hansson, Ö. and Vernotte, C. (1986) Photobiochem. Photobiophys. 13, 15–28.
16 Yamada, Y., Itoh, N. and Satoh, K. (1985) Plant Cell Physiol. 26, 1263–1271.
17 De Vitry, C., Wollman, F.-A. and Delepelaire, P. (1984) Biochim. Biophys. Acta 767, 415–422.
18 Conjeaud, H., Mathis, P. and Paillotin, G. (1979) Biochim. Biophys. Acta 546, 280–291.
19 Van Best, J.A. and Mathis, P. (1978) Biochim. Biophys. Acta 503, 178–188.
20 Schlodder, E., Brettel, K., Schatz, G.H. and Witt, H.T. (1984) Biochim. Biophys. Acta 765, 178–185.
21 Brettel, K., Schlodder, E. and Witt, H.T. (1984) Biochim. Biophys. Acta 766, 403–415.
22 Mathis, P. and Setif, P. (1981) Isr. J. Chem. 21, 316–320.
23 Fujita, I., Davis, M.S. and Fajer, J. (1978) J. Am. Chem. Soc. 100, 6280–6282.
24 Bowers, P.G. and Porter, G. (1967) Proc. R. Soc. A, 296, 435–441.
25 Van Gorkom, H.J. (1985) Photosynth. Res. 6, 97–112.
26 Sebban, P. and Barbet, J.C. (1984) FEBS Lett. 165, 107–110.
27 Woodbury, N.W. and Parson, W.W. (1986) Biochim. Biophys. Acta 850, 197–210.
28 Hörber, J.K.H., Göbel, W., Ogrodnik, A., Michel-Beyerle, M.E. and Cogdell, R.J. (1986) FEBS Lett. 198, 273–278.
29 Moya, I., Hodges, M., Briantais, J.-M. and Hervo, G. (1986) Photosynth. Res. 10, 319–325.
30 Murata, N., Araki, S., Fujita, Y., Suzuki, K., Kuwabara, T. and Mathis, P. (1986) Photosynth. Res. 9, 63–70.