Biochimica et Biophysica Acta, 934 (1988) 389–395
Elsevier

BBA 42805

Spectroscopic characterization of the Photosystem II chlorophyll-protein complexes

Y. Choquet [a], C. de Vitry [a], P. Delepelaire [a,*], F.A. Wollman [a] and P. Tapie [b]

[a] *Institut de Biologie Physico-Chimique, Paris and* [b] *ARBS CEN Cadarache, St Paul-lez-Durance (France)*

(Received 18 March 1988)

Key words: Photosystem II; Linear dichroism; Pheophytin; Pigment orientation; (*C. reinhardtii*)

We investigated by polarized light spectroscopy the organization of the pigments in the Photosystem II (PS II) reaction center of *Chlamydomonas reinhardtii* using PS II particles and subparticles of varying photochemical activity, antenna size and polypeptide composition. We demonstrate that the amplitude of the pheophytin photoreduction varies in parallel with the presence of a transition arising from the pheophytin molecules. We show that the transitions arising from the photoactive pigments are absent in the chlorophyll-protein complexes III and IV (CPIII and CPIV), and that all the other transitions present in the PS II particles are conserved, suggesting that CPIII is more likely to act as an antenna, as is the case for CPIV, rather than to bind alone the primary reactants.

Introduction

Contrasting with the bacterial reaction center, the identity of the polypeptides that bind the photosystem II (PS II) primary reactants is still a matter of controversy; two major models resulting from conflicting observations have been put forward.

In the first one, the primary donor (P-680) and the primary acceptor (pheophytin) are localized on chlorophyll-protein complex 47 (CP47). Indeed, CP47 and CP43, first designed CPIII and CPIV in *C. reinhardtii* [1], have been for a long time the only known chlorophyll-binding complexes in PS II. Each complex contains 4–5 Chl, one β-carotene and one polypeptide. The apoprotein of CPIII is protein 5 (p5) (50 kDa) and that of CPIV is polypeptide 6 (p6) (47 kDa). It was recently reported [2] that the removal from PS II particles of the two intrinsic membrane proteins with molecular masses close to 32 kDa, designed in *C. reinhardtii* as polypeptide D1 (the binding site of inhibitors blocking Q_a to Q_b electron transfer) and polypeptide D2, does not impair photoreduction of the primary quinonic acceptor (Q_a). Also consistent with this model is the report [3] that photoinhibited thylakoids largely devoid of D1 are badly damaged at the secondary quinonic acceptor (Q_b) site of PS II, but show few changes at the Q_a site as well as on the donor side of PS II.

In the second model, the association of D1 and D2 polypeptides is proposed to be the site of the primary charge separation in PS II, as is the case for the L and M subunits in purple bacteria. This was first suggested [4] by sequence homologies

* Present address: Unité de Génétique Moléculaire, Institut Pasteur, Paris, France.

Abbreviations: Chl, chlorophyll; CPIII and CPIV, chlorophyll-protein complexes III and IV; LD, linear dichroism; PS I and PS II, Photosystems I and II; p5 and p6, polypeptides 5 and 6; P-680, primary donor of the PS II; Q_a and Q_b, primary and secondary quinonic acceptors of the PS II.

Correspondence (present address): Y. Choquet, Department of Molecular Biology, University of Geneva, 1211 Geneva 4, Switzerland.

between D1 and D2 in the PS II reaction center [5,6] and the L and M subunits in the bacterial reaction center [7]. In particular, the histidine residues that ligand the bacteriochlorophyll special pair (the primary donor) and the non-heme iron in the bacterial reaction center of *Rhodopseudomonas viridis* are conserved in D1 and D2 [8]. Consistent with this model is the recent observation of a possible binding of chlorophyll molecules to PS II polypeptides in the 30–34 kDa region [9]. Furthermore, there has been one report that the isolation of a PS II complex which contains D1, D2 and cytochrome *b*-559 is devoid of CP47 and CP43 and still photoreduces the pheophytin [10].

The description of the arrangement of the pigment molecules inside the reaction center is an important step towards understanding the primary reactions. In this report, we analyzed by polarized light spectroscopy the organization of the pigments within the PS II reaction center. We isolated from *C. reinhardtii* PS II particles and subparticles of various photochemical activity, antenna size and polypeptide composition: PS II centers depleted of CPIV and still able to photoreduce the primary acceptor, pheophytin, and PS II subparticles CPIII and CPIV. To gain information about the structure of PS II photoactive pigments, we compared the pigment organization in PS II photoactive particles and in CPIII and CPIV devoid of photochemical activity. We attempted to correlate the amplitude of pheophytin photoreduction with the amplitude of one of the oriented transitions arising from the pheophytin.

Materials and Methods

CPIII and CPIV complexes were prepared as described in Ref. 1. PS II particles of different polypeptide compositions were isolated according to Ref. 11 from the following two strains of *C. reinhardtii*: (i) a double mutant strain, BF4 : M18, equivalent to the BF4 : 14 mutant strain previously described [12] lacking the PS I reaction center and largely deficient in PS I peripheral antenna and major antenna complex (LHC); (ii) a double mutant strain, 54 : 14, lacking the PS I reaction center and ATPase [13]. Pheophytin activity measurements and determination of polypeptide composition of the complexes were performed according to Ref. 11.

The protein-pigment complexes were oriented by the polyacrylamide gel squeezing method and absorption, linear dichroism (LD) and polarized fluorescence emission spectra were recorded at low temperature (5 or 100 K) as described in Ref. 14 *. The experimental arrangement allows a very high resolution of LD data ($LD = A_{\parallel} - A_{\perp}$) because of direct recording of $A_{\parallel} - A_{\perp}$, while fluorescence spectra, measured as $F_{\parallel}$ and $F_{\perp}$ separately, do not bear the same accuracy in the $F_{\parallel} - F_{\perp}$ value. The LD and fluorescence spectra presented here are normalized to a value of 1.0 for the amplitude at the red maximum.

We determined the relative amount of oriented pheophytin per chlorophyll molecule by calculating the ratio of the area under the 543 nm LD band (533–553 nm) versus the area under the absorption red band (640–690 nm). Both calculations were performed on the recorded spectra, i.e., before normalization. The observed orientation of pigments results from two contributions: the orientation of the pigments within protein-pigment complexes and the macroscopic orientation of the complexes inside the gel. These two phenomena are quantified by the formula:

$$LD/A = \sigma_a = S_a \cdot \phi$$

where ϕ is the mosaic spread parameter which describes the degree of orientation of the collec-

* The principle of the method is as follows: Uniaxial orientation of anisotropic complexes is achieved by the polyacrylamide gel squeezing method [15] and absorption or fluorescence parallel or perpendicular to the squeezing direction are recorded. Polarized light spectroscopy allows investigation of orientation of absorbing or fluorescing dipoles within this anisotropic, oriented sample. It has been previously shown [16] that in the gel, the complexes behave like ellipsoids in which the pigments exhibit an orientation identical to the one they have in the native thylakoid membrane. Accordingly, in the following discussion, the orientation of the pigments will always be defined with respect to a plane which is either the plane of the largest cross-section of the ellipsoid in the case of an isolated complex or the plane of the membrane in the case of intact thylakoids. Therefore, a positive LD signal indicates an absorbing dipole tilted more than 55° from the normal plane of the membrane (for a review, see Ref. 17).

tion of particles and S_a is the order parameter, related to the angle θ between a transition and the normal to the plane of a particle by the formula: $S_a = (3\cos^2\theta - 1)/2$ [18]. Assuming a similar orientation of the 676 nm transition (thus an identical value for $S_a(676)$ parameter) inside every PS II particle studied, measurements of oriented pheophytin were corrected for variations of the mosaic spread factor ϕ by dividing the calculated value (orange LD band versus red absorption band) by $\sigma_a(676)$.

Results and Discussion

Correlation between oriented and photoactive pheophytin

The specific distance and orientation of the pigments, and particularly of the photoactive ones, inside the chlorophyll-protein complexes are highly critical in the process of energy conversion in the photochemical reaction centers. It is therefore of interest to establish correlations between photoactivity and oriented signals arising from these pigments. As the LD spectrum of a PS II particle in the 'red' range (630–710 nm) is complex and made of several overlapping transitions of various orientations, a quantitation of most signals attributed to photochemically active pigments is difficult. We decided to focus on the oriented transition LD_{543}, clearly resolved from the background and attributed to pheophytin [14,16,19]. It has been shown that PS II particles contain two pheophytins per P-680 [20], only one of them being active in photoreduction [21]. It is likely that both pheophytins contribute to the LD_{543} signal. This positive peak at 543 nm indicates that pheophytin is tilted less than 35° from the membrane plane. We have studied various preparations of PS II particles more or less depleted in CPIV and containing from 0.68 to 2 active pheophytins per 48 Chl molecules [11] (see Table I). Their LD spectra recorded at 5 K were very similar to PS II spectra already obtained for intact PS II particles [14,22]. Fig. 1 and Table I show that the amplitude of the pheophytin photoreduction (designed as active pheophytin) and the presence of the pheophytin transition at 543 nm (designed as oriented pheophytin) are roughly linearly correlated. The two points with higher pheophytin enrichment are shown as extreme values (two active pheophytins per 48 Chl would correspond to a particle without any CPIV: $p5/(p5 + p6) = 1$); they have been excluded from the regression because the corresponding particles showed some denaturation of antenna pigments absorbing at 670 nm. This resulted in the underestimation of the $\sigma_a(676)$ factor leading to an overestimation of the oriented pheophytin amount. The fact that the obtained regression crosses the axes near the origin indicates that LD_{543} is associated with the primary charge separation and that the orientation of the pheophytins is conserved when the PS II particles loose the CPIV.

TABLE I

PS II CONTENT IN CPIII AND CPIV APOPROTEINS (p5 AND p6) AND IN ACTIVE AND ORIENTED PHEOPHYTIN

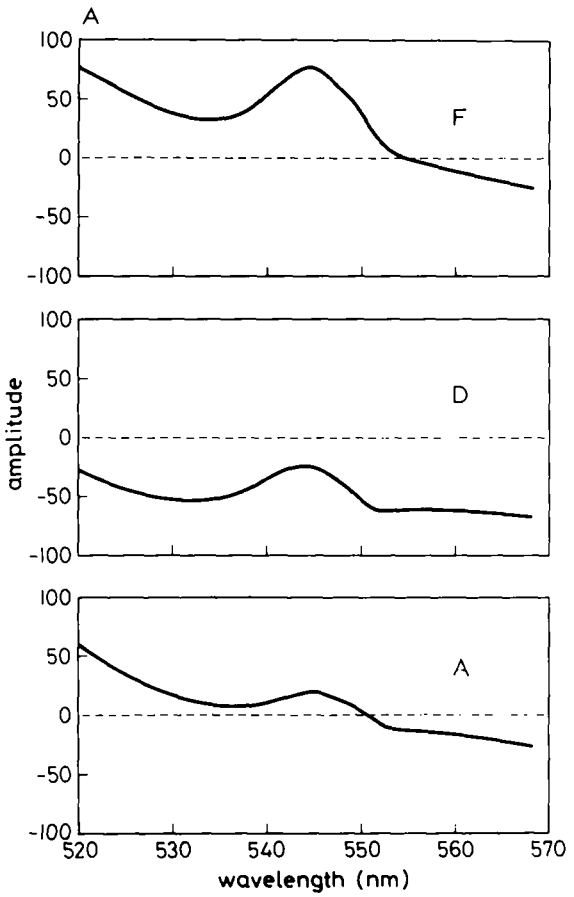
A,C and B,D samples were prepared from F54:14 and BF4:M18 strains, respectively, according to Ref. 13, E,F samples were prepared from BF4:M18 strain according to a modified Mullet procedure [11]. Polypeptide composition was expressed as the molar ratio of polypeptide 5. Active pheophytin is expressed on the basis of 48 Chl. The amount of oriented pheophytin per chlorophyll was determined as described in Materials and Methods and normalized to sample B, which contains 1 active pheophytin per 48 Chl.

Sample	$\frac{p5}{p5+p6}$	Active pheophytin per 48 Chl	Oriented pheophytin per Chl
A	0.52	0.68	0.75
B	0.58	1.00	1.00
C	0.62	1.04	0.83
D	0.71	1.20	1.25
E	0.75	1.65	2.38
F	0.83	2.00	3.05

These PS II particles, although they are enriched in oriented (or active) pheophytin, show no F_{695} fluorescence emission (data not shown). Therefore, we can rule out that F_{695} directly originates from the main transition of the oriented pheophytin.

Structural spectroscopic characterization of CPIII and CPIV compared to photoactive PS II particles

As shown in Table I, the particles with the higher pheophytin activity are more depleted in



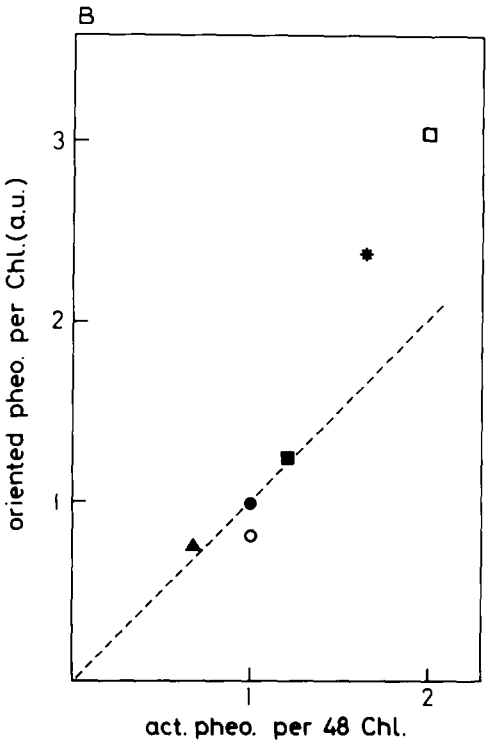


Fig. 1. (A) Amplified LD spectra in the 'orange' region (520–570 nm) of three different samples of PS II particles isolated from *C. reinhardtii* (see Table I) with different pheophytin activity. Spectra were recorded at 5 K and have been normalized to an equal value at the LD red peak maximum for ease of comparison. (B) The correlation between the amount of oriented pheophytin per chlorophyll molecule and the pheophytin photoreduction activity determined for six different particles characterized in Table I (A, ▲; B, ●; C, ○; D, ■; E, *; F, □).

CPIV. Thus, as previously reported [11], CPIV does not participate in the binding of the pheophytin and this confirms its role of core antenna devoid of photoactivity. As the relative amount of D1 and D2 has not been monitored in these experiments, their participation in the binding of the primary reactants could not be assessed. However, further information on the orientation of the pigments close to the PS II reaction center can be obtained by comparing intact PS II particles with purified CPIII and CPIV complexes. This should show whether CPIII and CPIV are both acting as core antennae, or whether CPIII contains transitions arising from photoactive pigments.

Despite their small size, CPIII and CPIV complexes do present a macroscopic orientation in squeezed polyacrylamide gels and they show quite reproducible LD and absorption spectra at 100 K (Figs. 2 and 3). However, the orientation of the complexes is weak (in CPIII and CPIV, $\sigma_{a(max)}$ is close to 0.01, while in PS II particles $\sigma_{a(max)}$ is close to 0.06) which precluded the measurement of the polarization of the fluorescence emission. These fluorescence emission spectra are both composed of a single band at 685 nm (Fig. 5). Although denaturation and partial reorganization of the pigments during the extraction of the complexes could have occurred, the existence of a fluorescence peak at the same wavelength in the

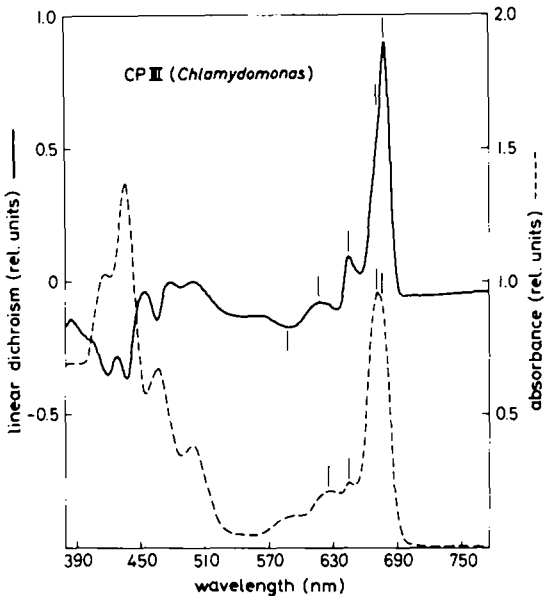


Fig. 2. Absorption (- - - - - -) and LD (———) of CPIII complexes. The spectra were recorded between 380 and 780 nm at 100 K on complexes oriented in squeezed polyacrylamide gels. The spectra were normalized to an arbitrary value; the maximum order parameter is about 10^{-2}.

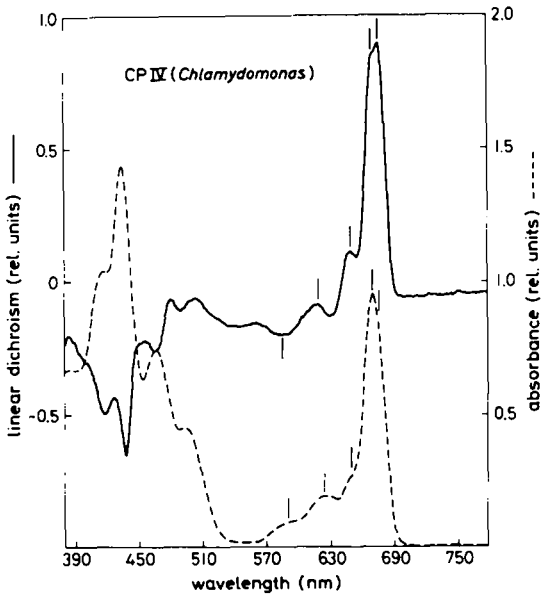


Fig. 3. Absorption (- - - - - -) and LD (———) of CPIV complexes. The spectra were recorded between 380 and 780 nm at 100 K on complexes oriented in squeezed polyacrylamide gels. The spectra were normalized to an arbitrary value; the maximum order parameter is about 10^{-2}.

photochemically inactive CPIII and CPIV particles as in native PS II complexes indicates a conserved pigment structure and strengthens the assertion that the LD_{676} component (PS II core antenna pigment) participates in the emission of F_{685} in photochemically active particles [14].

In the spectral range 630–710 nm, where the Q_y transitions of the chlorophylls are involved, the LD is positive, and, except for minor transitions related to photochemical activity (see below), the general shapes for both LD and absorption spectra are similar in CPIII and CPIV which contain 4–5 Chls and in PS II particles binding 50 Chls (see Refs. 14, 22 for spectra). Thus, CPIII and CPIV (the state of aggregation of these complexes in the gel has not been investigated) have the same macroscopic orientation in the gel with respect to the squeezing direction as they have in native PS II particles and in the membrane.

Absorption and LD spectra of these two complexes show four major components at 645, 665, 670 and 676 nm. The 682 nm component de-

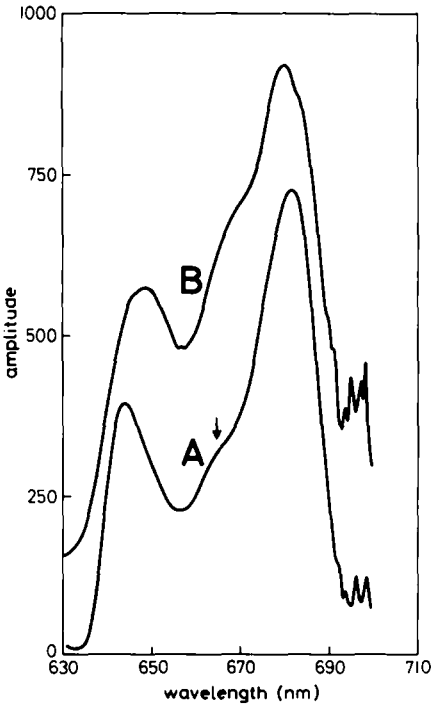


Fig. 4. LD/*A* spectra of the CPIII (A) and CPIV (B) complexes amplified in the red region (630–690 nm). The vertical arrow indicates the LD_{665} component in the CPIII spectra.

scribed by Delepelaire and Chua [1] in CPIII is very weak in our spectra (Fig. 2a and 2b). The 645 nm component is due to a pigment specific to *C. reinhardtii* and other algae [23] already described [14], and strongly bound to both polypeptides 5 and 6. As previously suggested, this component is not due to Chl *b*, since CPIII and CPIV contain only negligible amounts of Chl *b* (De Vitry, C., unpublished results). The 665 nm component is more abundant in CPIV particles but can be seen in the LD and LD/*A* spectra of CPIII particles (Fig. 4). The 670 nm component is the major component in absorption spectra. This transition, tilted at an angle close to 35° from the membrane plane, only gives a small LD signal. The 676 nm component is the one oriented closest to the membrane plane (maximum of the LD/*A* spectra) in CPIII and CPIV. In the spectral range 530–630 nm, the broad negative transition, typical of PS II complexes and tentatively ascribed to the Q_x transition of Chl *a*, is also present in our spectra.

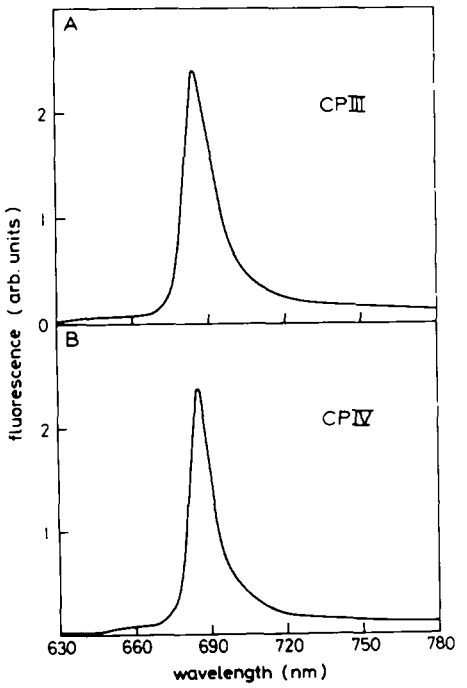


Fig. 5. Fluorescence emission spectra of CPIII (A) and CPIV (B) complexes. The spectra were recorded between 630 and 780 nm in liquid nitrogen on complexes oriented in squeezed polyacrylamide gel.

These 645, 665, 670, 676 nm-absorbing chlorophylls are therefore bound in a very similar way to both polypeptide 6, known as an antenna without photochemical activity [11,24,25], and polypeptide 5. This similarity between CPIII and CPIV spectra is not surprising, since homologies have been reported between the sequences of the two apoproteins. These homologies are especially significant in the presumed intramembrane spanning domains, where there is a high conservation of the histidine residues presumed to interact with the chlorophylls [26,27].

Interestingly, CPIII and CPIV spectra, when compared to the spectra of photoactive PS II particles, are missing all of the signals previously ascribed to the PS II primary reactants (the 543 nm, 681 nm (pheophytin), 683 nm (P-680), 693 nm (unknown origin) components [14]). This is consistent with the absence of any photochemical activity in CPIII and CPIV complexes and supports further the correlation between the photoactivity and the proper orientation of the transitions arising from primary reactants. Except for these signals, all the other transitions present in spectra of PS II particles are also present in CPIII and CPIV spectra at the same wavelengths and with similar orientations. Thus, the organization of pigments in CPIII and CPIV is indicative of the organization of the PS II antenna pigments. Several conclusions can be drawn from the similarity between the LD spectra of CPIII, CPIV and PS II particles binding 50 chlorophylls: (i) At least part of the 645, 665, 670, 676 nm-absorbing chlorophylls (already ascribed to PS II core antenna [14]) must lie in PS II complexes in an internal position, highly protected from the detergent, since the structural arrangement of these chlorophylls is unaltered in CPIII and CPIV. (ii) The PS II antenna chlorophylls that have been lost during CPIII and CPIV isolation are oriented in PS II particles as are the chlorophylls that remain in CPIII or CPIV. This appears highly surprising, as the remaining pigments correspond only to one quarter of those bound to the PS II complex. Further structural information on the folding of the PS II subunits is required to interpret this observation.

The fact that all the transitions ascribed to the PS II primary reactants are missing in CPIII spec-

tra rules out the possibility that CPIII would bind the primary reactants in an inactive form. Therefore, it is unlikely that polypeptide 5 alone would bind the primary reactants. Given the high structural homology between CPIII and CPIV complexes, our results suggest that CPIII is more likely to act as antenna, as in the case for CPIV. However, whether the binding sites of the primary reactants are built up by the interaction between the polypeptide 5, D1 and D2 [11,24,25] or only by the latter two polypeptides remains to be determined.

Acknowledgment

This work was supported by the Agence Française pour la Maîtrise de l'Energie (fellowship to C. de V.).

References

1 Delepelaire, P. and Chua, N.-H. (1979) Proc. Natl. Acad. Sci. USA 76, 111–115.
2 Satoh, K. (1986) FEBS Lett. 204, 357–362.
3 Kyle, D.J., Ohad, I. and Arntzen, C.J. (1984) Proc. Natl. Acad. Sci. USA 81, 4070–4074.
4 Michel, H. and Deisenhofer, P. (1986) in Encyclopedia in Plant Physiology: Photosynthesis III (Staehelin, A.C. and Arntzen, C.J., eds.), pp. 371–381, Springer. Berlin.
5 Erickson, J.M., Rahire, M. and Rochaix, J.D. (1984) EMBO J. 3, 2753–2762.
6 Rochaix, J.D., Dron, M., Rahire, M. and Malnoe, P. (1984) Plant Mol. Biol. 3, 363–370.
7 Michel, H., Weyer, K.A., Gruenberg, H., Dunger, I., Oesterhelt, D. and Lottspeich, F. (1986) EMBO J. 5, 1149–1158.
8 Michel, H., Epp, O. and Deisenhofer, J. (1986) EMBO J. 10, 2245–2251.
9 Irrgang, K.-D., Renger, G. and Vater, J. (1986) FEBS Lett. 204, 67–75.
10 Nanba, O. and Satoh, K. (1987) Proc. Nat. Acad. Sci. USA 84, 109–112.
11 De Vitry, C., Wollman, F.A. and Delepelaire, P. (1985) Biochim. Biophys. Acta 767, 415–422.
12 Wollman, F.A. (1982) Thesis, Université Paris VII.
13 Diner, B. and Wollman, F.A. (1980) Eur. J. Biochem. 110, 521–526.
14 Tapie, P., Choquet, Y., Wollman, F.A., Diner, B. and Breton, J. (1986) Biochim. Biophys. Acta 850, 156–161.
15 Haworth, P., Tapie, P., Arntzen, C.J. and Breton, J. (1982) Biochim. Biophys. Acta 682, 152–159.
16 Tapie, P., Haworth, P., Hervo, G. and Breton, J. (1982) Biochim. Biophys. Acta 682, 339–344.
17 Breton, J. and Vermeglio, A. (1983) in Energy Conversion by Plants and Bacteria (Govindjee, ed.), pp. 153–194, Academic Press, New York.
18 Tapie, P., Choquet, Y., Delepelaire, P. and Wollman, F.A. (1984) Biochim. Biophys. Acta 767, 57–69.
19 Ganago, I.B., Klimov, V.V., Ganago, A.O., Shuvalov, V.A. and Erohin, Y.U. (1982) FEBS Lett. 140, 127–130.
20 Omata, O., Murata, N. and Satoh, K. (1984) Biochim. Biophys. Acta 765, 403–405.
21 Klimov, V.V., Klenavic, A.V., Shuvalov, V.A. and Krasnovsky, A.A. (1977) FEBS Lett. 82, 183–186.
22 Tapie, P., Acker, S., Arntzen, C.J., Choquet, Y., Delepelaire, P., Diner, B., Wollman, F.A. and Breton, J. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 693–696, Martinus Nijhoff/Dr. W. Junk, Dordrecht.
23 Thomas, J.B. (1972) Proc. 2nd Int. Cong. Photosyn. Res. 1509–1513.
24 Yamagishi, A. and Satoh, K. (1985) Biochim. Biophys. Acta 807, 74–80.
25 Takahashi, Y. and Katoh, S. (1986) Biochim. Biophys. Acta 848, 183–192.
26 Halt, J., Morris, J., Westhoff, P. and Hermann, R.G. (1984) Curr. Genet. 8, 597–606.
27 Holschuh, K., Bottomley, W. and Whitfeld, P.R. (1984) Nucleic Acids Res. 12, 8819–8833.