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Orientation of the pigments in Photosystem II: low-temperature linear-dichroism study of a core particle and of its chlorophyll-protein subunits isolated from *Synechococcus* sp.

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The orientation of the pigments in the Photosystem II core particle isolated from the thermophilic cyanobacterium *Synechococcus* sp. has been investigated by linear dichroism spectroscopy at 10 K of macroscopically oriented samples. The absorbance (A), linear dichroism (LD) and LD/A spectra are remarkably similar to those previously reported for a core complex isolated from *Chlamydomonas reinhardtii* (Biochim. Biophys. Acta 850 (1986) 156–161). The spectra of the *Synechococcus* core particle are compared to the corresponding spectra obtained on its two main constituent chlorophyll-protein complexes CP2-b (photochemically active) and CP2-c (photochemically inactive). The various features seen in the spectra of the core particle appear well segregated into the spectra of one or the other of the two subparticles without significant loss of orientation of the pigments. The orientation of the chlorophyll macrocycles, with the *Y* and *X* optical axis preferentially parallel and perpendicular to the plane of largest cross-section of the particle, respectively, is very similar in the two subparticles. CP2-b contains mainly the beta-carotene pool absorbing around 505 and 470 nm, which is oriented close to the membrane plane, while CP2-c contains the beta-carotene pool absorbing around 495 and 465 nm and oriented closer to the normal to the membrane plane. A shoulder at 682 nm in the absorbance and linear dichroism spectra of the core complex is fully segregated in the spectra of CP2-c, thus excluding the possibility that this spectral feature could be assigned to the primary donor of PS II. A negative linear dichroism component peaking around 691 nm (LD 691) in the core particle is mainly segregated in CP2-b together with the photoactive pheophytin acceptor molecule responsible for the 544 nm positive linear dichroism signal (LD 544). While the ratio of the amplitudes LD 691/LD 544 is approximately the same for the core particle and for the CP2-b complex, the amplitude of LD 691 is significantly reduced in CP2-b compared to the core particle.

Introduction

The primary photochemistry occurring in the Photosystem II (PS II) of green plants, algae and cyanobacteria is currently thought to involve a charge separation between the primary donor chlorophyll(s) of P-680 and a pheophytin accep-

Abbreviations: PS II, Photosystem II; P-700, primary donor of Photosystem I; P-680, primary donor of Photosystem II; ADMR, absorbance-detected magnetic resonance.

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tor. This event is followed by a stabilization step where the electron moves from the pheophytin to the first quinone acceptor (Q_A). With respect to these primary reactions, PS II bears striking analogies with purple photosynthetic bacteria [1]. In purple bacteria, the isolation and purification of a functional reaction center complex lacking its associated antenna has greatly facilitated our understanding of the structural and energetic factors governing these primary reactions. While the spatial organization of the bacterial reaction center chromophores has been inferred from various optical and EPR spectroscopic measurements, the recent crystallographic determination of the structure of the reaction center from *Rhodopseudomonas viridis* at 0.3 nm resolution has provided an invaluable picture of the general organization of the constituent pigments and proteins [2].

In the case of PS II the purification of a minimal reaction center comparable to that of photosynthetic purple bacteria has not yet been achieved and the photochemical activity is associated with a specific chlorophyll-protein complex bearing typically 40 chlorophyll *a* per P-680. Such PS II particles, which contain the functional components involved in the charge separation and stabilization steps, consist of five protein subunits with apparent molecular weights of about 47, 40, 30–34 and 9–10 kDa [4–11]. The two large polypeptides of 47 and 40 kDa carry antenna chlorophyll *a*, whereas the 9–10 kDa polypeptide constitutes the apoprotein of cytochrome *b*-559. The two polypeptides in the 30–34 kDa range are named D_1 and D_2 and the former has been associated with herbicide-binding activity [6]. In view of the analogies in the primary sequences of the D_1 and D_2 polypeptides with those of the L and M subunits, which constitute the scaffold of transmembrane alpha-helices holding together the essential cofactors in the reaction center of purple bacteria, it has been suggested that D_1 and D_2 play a similar role in forming the reaction center of PS II [12,13]. Several laboratories have reported the fractionation of PS II particles from various organisms into two smaller chlorophyll-protein complexes containing one or the other of the two large polypeptides [4–11]. In general, the PS II activity, although significantly reduced compared to that of the native particles, was found in association with the complex carrying the largest polypeptide [5,7,10]. Using a thermophilic strain of the cyanobacterium *Synechococcus*, Yamagishi and Katoh have been able to isolate and characterize two such subparticles, named CP2-b and CP2-c, which exhibit a markedly increased stability compared to that of their counterparts isolated from green plants and algae [7–9]. CP2-c contains only the 40 kDa chlorophyll *a*-binding polypeptide, is lacking pheophytin and exhibits no PS II activity, while CP2-b which contains the 47, 30–34 and 9–10 kDa polypeptides but not the 40 kDa subunit, is able to photoreduce Q_A and to photoaccumulate reduced pheophytin [9].

Recently, the organization of the pigments in small unfractionated PS II particles from *Chlamydomonas* has been investigated by polarized light absorption and emission spectroscopies at cryogenic temperatures [14]. Such studies can reveal useful relationships between the presence of some spectral components, the polypeptide composition and the biological function associated with each of these particles. Amongst the various observations reported in this study, a correlation was established between the magnitude of a small spectral component around 693 nm in the 5 K linear dichroism spectra and the amplitude of the characteristic PS II emission band at 695 nm (F 695). Because the orientation of the F 695 emission, rather perpendicular to the plane of the thylakoid membrane [15–17], is both different from that observed for the Q_Y absorption and emission dipoles of most of the chlorophylls in vivo and identical to the orientation of the Q_Y transition of the pheophytin acceptor [18], it has been hypothesized that F 695 was emitted directly from this last molecule [19]. The negative linear dichroism signal around 693 nm [14] or 692 nm [20] corresponding also to dipoles oriented rather perpendicular to the membrane plane, it has been further proposed that this spectral feature could also be assigned to this pheophytin molecule [21]. The 77 K fluorescence properties of the PS II subparticles from *Synechococcus* sp. have been characterized [7]. CP2-b has been shown to emit maximally at about 695 nm, while CP2-c emits around 685 nm. Using these two subparticles it becomes possible to compare for the first time the absorption and linear dichroism at Helium tem-

perature of the native PS II particles to those of its two main constituent chlorophyll-protein complex.

In the present work we compare the linear dichroism of the core particle to the sum of the linear dichroism of the two subparticles. We determine in which of these two subparticles the 682-nm shoulder tentatively assigned to P-680, the different pools of carotenoid molecules and the negative linear dichroism component at about 692 nm are segregated [14] and we further assess for the proposed origin of F 695.

Materials and Methods

Synechococcus sp. was grown at 55°C for 2 days [22] and the thylakoid membranes were prepared from mechanically disrupted protoplasts as in Ref. 23. The PS II reaction center particles were isolated as described in Ref. 8 with a modification in the acrylamide concentration of gels. In short, the thylakoid membranes were first treated with 0.4% beta-octylglucoside at 0°C for 30 min and pelleted by centrifugation at 25 000 × *g* for 20 min to remove allophycocyanin bound to the membranes. The membranes were then solubilized with 0.8% beta-octylglucoside at 25°C for 30 min and, after centrifugation at 230 000 × *g* for 20 min, the green supernatant was subjected to electrophoresis in a gel containing 5% acrylamide and 0.2% digitonin. To prepare the PS II particles lacking the 40 kDa polypeptide (CP2-b) and the chlorophyll-protein complex containing only the 40 kDa polypeptide (CP2-c), the membranes were extracted with 0.3% lauryldimethylamine *N*-oxide at 0°C for 30 min and the supernatant obtained by centrifuging the extracts at 230 000 × *g* for 40 min was applied to a 7.5% acrylamide gel with 0.05% SDS present in the reservoir buffer but not in the gel. The PS II particles and the smaller complexes were extracted from homogenized gels with 50 mM Tris-HCl (pH 7.5), concentrated by centrifugation, suspended in 0.5 M sucrose containing 50 mM Tris-HCl (pH 7.5) and stored at liquid nitrogen temperature (the frozen samples were shipped by air to France in dry ice). Polypeptide composition of the three preparations were essentially the same as those reported in Ref. 8, except that the undissociated particle contained trace amounts of allophycocyanin subunits. No phycobiliproteins were detected in CP2-b and CP2-c.

The orientation of the samples in squeezed polyacrylamide gels as well as the recording of the linear dichroism and absorbance spectra at Helium temperatures have been previously described [14,24,25].

Results

The absorption (A), linear dichroism (LD) and LD/A spectra at 10 K of the undissociated PS II particle from *Synechococcus* sp. (Figs. 1a and 2a) are quite comparable to the corresponding spectra recently reported for the PS II particle from *Chlamydomonas* [14]. In the red spectral range (Fig. 2a) they show an absorption peak at 669 nm with shoulders at 673, 678 and 682 nm, a linear dichroism maximum (positive) at 674 nm with shoulders at 665, 670, 678 and 682 nm and a pronounced negative linear dichroism component at 691 nm. A shoulder around 655 nm in the absorption spectra which exhibits a variable amplitude from sample to sample is ascribed to a small amount of allophycocyanin. When comparing the linear dichroism spectra of particles with variable amounts of this 655 nm-absorbing component (data not shown), it can be concluded that the dipole of this terminal bilin excitation energy acceptor is oriented preferentially parallel to the plane of the particle. In the 550-nm region small but reproducible absorption and linear dichroism signals are observed leading to well-defined features in the LD/A spectra which indicate a positive linear dichroism at 544 nm and an S-shaped signal centered at 556.5 nm. In the blue spectral range a shoulder around 510 nm in the long-wavelength absorption band of the carotenoid is associated with a positive linear dichroism, while most of the carotenoid absorption bands at 465 and 495 nm are associated with negative linear dichroism signals. The peak of the chlorophyll *a* Soret band at 435 nm is associated with a distinct negative linear dichroism signal. Most of these features present in the spectra of the PS II particles from *Synechococcus* sp. have been already observed in the spectra of PS II particles from other organisms [14,20].

The absorbance, linear dichroism and LD/A

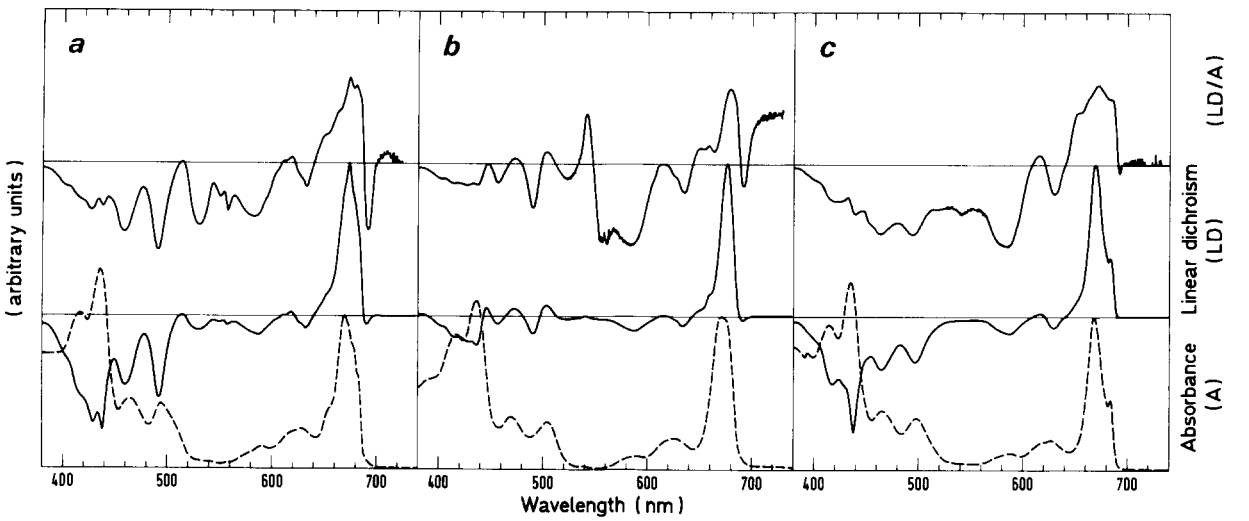

Fig. 1. Absorbance (A), linear dichroism (LD) and LD/A spectra of (a) the PS II core complex, (b) the photochemically active CP2-b and (c) the photochemically inactive CP2-c subparticles isolated from the thermophilic cyanobacterium *Synechococcus* sp. The particles were oriented in squeezed polyacrylamide gel and the spectra were recorded at 10 K. All the absorption and linear dichroism are normalized to an arbitrary value at their maximum in the red spectral range. The dichroic ratios at the 670–675 nm maximum at room temperature were 1.3, 1.4 and 1.25 for the samples a, b and c, respectively.

spectra of CP2-b (Figs. 1b and 2b) are significantly different from the corresponding spectra of both the CP2-c complex (Figs. 1c and 2c) and the intact PS II particle (Figs. 1a and 2a). The spectra of the two subparticles show a much simpler structure with less spectral components than in the intact particle. While most of the spectral features seen in the intact system can be observed in the spectra of either one of the smaller complexes, this is not true for all the components. More specifically, the 655 nm shoulder seen in Fig. 1a is almost absent from the spectra of CP2-b and CP2-c, reflecting the removal of allophycocyanin during preparation of the complexes by SDS gel electrophoresis. The same situation also applies to the linear dichroism signals located

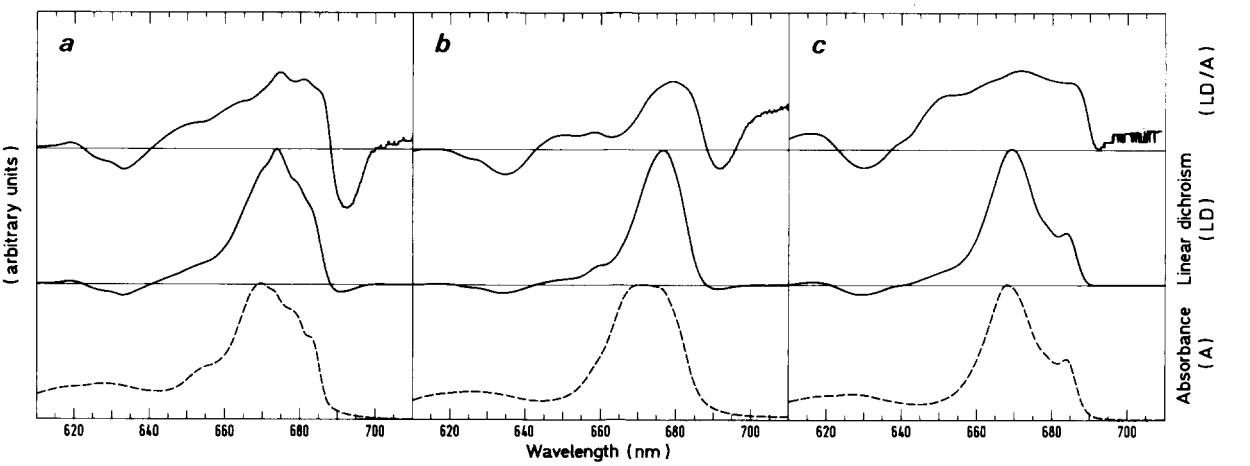

Fig. 2. Same conditions as for Fig. 1, but the 610–710 nm spectral range has been expanded.

around 556.5 and 430 nm, which may be ascribed to absorption bands of reduced cytochrome *b*-559. A fraction of the cytochrome was present in the reduced state in the PS II particles, whereas CP2-b contained only the oxidized cytochrome as SDS converts cytochrome *b*-559 into its low potential form [9]. Apart from these two spectral components, however, the features seen in the spectra of the intact PS II particle appear well segregated in the spectra of one or the other of the two smaller complexes. Notably, the negative linear-dichroism component at 691 nm appears almost exclusively in CP2-b, while the 682 nm shoulder, to which is associated a positive linear dichroism, is entirely segregated in CP2-c. We note that the presence of a shoulder at 682 nm had been previously observed in the 77 K absorption spectra of subparticles of PS II having a similar composition in the 40 kDa polypeptide [4,7]. The absorption, linear dichroism and LD/A spectra of CP2-c also include a peak at 669 nm and a small shoulder at about 678 nm (both with a positive dichroism). The LD/A is rather flat in the region 660–685 nm due to the remarkable resemblance between the linear dichroism and absorption spectra in the red spectral range (Fig. 2c). A very different situation is observed for CP2-b which exhibits a rather flat absorption maximum extending for about 10 nm around 670 nm, but whose narrow positive linear dichroism signal peaks around 676 nm on the long-wavelength side of the flat absorption maximum (Fig. 2b). Together with the negative LD 691 signal, this property is responsible for the large variations of the amplitude of the LD/A spectrum in the red spectral range. In the blue spectral region the most noticeable difference in the absorption spectra of CP2-b and CP2-c is the position of the absorption maxima of the carotenoids, which are shifted about 10 nm to the red in CP2-b compared to CP2-c. The linear dichroism and LD/A spectra of the CP2-c subparticle indicate that the carotenoid population has a rather homogeneous orientation, with a negative linear dichroism. The situation is different in CP2-b where the largest pool of carotenoids, absorbing at 505 and 470 nm, is oriented preferentially close to the membrane, while a smaller fraction, oriented at a very large angle from the membrane, is responsible for the negative linear dichroism signals at 490 and 455 nm. Noticeable also is the large negative dichroism associated with the peak of the Soret band in CP2-c, while a smaller negative linear dichroism is observed in the same region for CP2-b.

Discussion

The high resolution which can be achieved when absorption and linear dichroism spectroscopies are performed at cryogenic temperatures is instrumental in demonstrating the remarkable similarities in the general organization of the pigments in the native PS II reaction center chlorophyll-protein complex isolated from different organisms such as spinach [20], *Chlamydomonas* [14,20] and *Synechococcus* (this study). On the other hand, the spectra shown in Figs. 1 and 2 clearly indicate large differences in the organization of the pigments in the two smaller complexes CP2-b and CP2-c. Although common features (such as the sign of the linear dichroism: positive in the red, negative in the Soret region) appear in all these spectra, there is the same level of differences in the linear dichroism spectra of CP2-b and CP2-c as there is between the spectra of a core PS II particle [21] and of the main light-harvesting antenna complex [24]. In general, the linear dichroism and absorption spectra of each of the two subparticles (Figs. 1b, 1c, 2b and 2c) appear significantly simpler (less spectral components) than the spectra of the core particle (Figs. 1a and 2a). This is especially remarkable in CP2-c, where the absorption and linear dichroism spectra have a very close resemblance in the red and in the blue (after inversion of the sign of the linear dichroism) spectral regions. Furthermore, the various features seen in the spectra of the core PS II particle (Figs. 1a and 2a) appear in the spectra of either one of the subparticles. This is also evident when comparing the spectra of the intact particle to the corresponding sum of the spectra of the two smaller complexes (Fig. 3a and 3b). Although not perfect, this agreement is remarkably good in view of the difficulties inherent in such adjustments which do not take into account the wavelength shifts induced by the presence of the charged SDS detergent in the smaller complexes [26]. Apart from the cytochrome *b*-559, the two other spectral

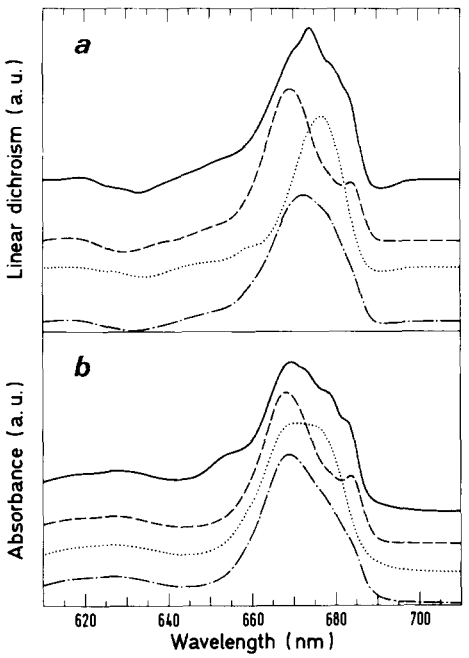

Fig. 3. Absorbance (b) and linear dichroism (a) spectra at 10 K of the core complex (———), the CP2-b (· · · · · ·) and CP2-c (— — —) subparticles. ·-·-·, linear combination of the spectra of the CP2-b (50%) and CP2-c (50%) subparticles.

features for which this agreement is lacking are (i) the 655-nm component assigned to the allophycocyanin present in the intact particle, but absent in the two subparticles and (ii) the amplitude of the 691-nm linear-dichroism signal relative to the amplitude at the red maximum, which will be discussed later. Apart from these features, it should be recognized that the calculated spectra resemble much more closely the spectra of the intact particle than those of the two smaller subparticles. In fact, the calculated spectra with their rather smooth shoulders very closely resemble the spectra of a core PS II particle measured at 100 K (Ref. 20; see also Breton, J., unpublished results) and it appears as if the SDS treatment used to split the two subparticles had slightly loosened or altered the binding sites of the pigments. The observation that the general shape of the linear dichroism and absorption spectra of the intact PS II particle can be closely mimicked by the algebraic sum of the corresponding spectra of CP2-b and CP2-c clearly implies that the orientation axis of these complexes in the gel is the same whether they are attached to or separated from each other. In previous studies [14,20,27] we have demonstrated that the isolated intact PS II particles behave in the gel like flattened ellipsoids, the normal of which (oriented along the gel-squeezing direction) coincided in vivo with the normal to the thylakoid plane. This is further verified in the present study using for example the characteristic S-shape of the cytochrome *b*-559 linear dichroism signal with a negative lop at 558 nm and a positive one at 554 nm, which is identical for the native particle (Fig. 1a) and for the intact thylakoids [28]. We thus conclude that in the gel the isolated CP2-b and CP2-c complexes adopt the same orientation with respect to the squeezing direction as they do when they are still embedded in the photosynthetic membrane. Furthermore, while the use of a one-to-one contribution of CP2-b and CP2-c to calculate the absorption spectrum is justified by the similar chlorophyll *a* content of the two subparticles [9], the identical coefficients used for the two normalized linear-dichroism spectra to obtain a satisfactory fit of the linear dichroism spectrum of the intact complex most probably indicate a rather homogeneous orientation of the chlorophylls in the two subparticles.

The absorption and linear dichroism spectra of the three PS II chlorophyll-protein complexes investigated in the present study (Fig. 1a, b and c) are remarkably similar in the spectral range 560–640 nm with peaks and shoulders at almost identical positions. This region can be ascribed to the vibrational transitions $Q_{YO\text{-}1}$ (around 620 nm) of the main $Q_{YO\text{-}0}$ bands (around 670 nm) and to the $Q_{XO\text{-}0}$ (around 630 nm) and $Q_{XO\text{-}1}$ (around 585 nm) transitions. The positive dichroism of the Q_Y transitions and the negative dichroism of the Q_X transitions demonstrate that at least most of the 15–20 chlorophyll *a* per CP2-b or CP2-c complex are oriented in vivo with their *Y* axis along the membrane plane and their *X* axis preferentially along the membrane normal. This type of orientation has been shown to be the general case for chlorophylls and bacteriochlorophylls in vivo [21,29] and it has been further proposed that transmembrane alpha-helices to which the pigments are bound were responsible for this type of orientation [30]. In this respect it is of interest to mention that transmembrane alpha-helical

stretches have been proposed from the primary sequence of the 47 kDa [31], of the 40 kDa [32] and of the D_1 [33] and D_2 [34] polypeptides. The large difference seen in the shape of the linear dichroism spectra of CP2-b and CP2-c at the peak of the Soret band is most probably not due to a difference in the orientation of the chlorophyll *a* molecules, but rather seems related to the opposite sign of the contribution of the oriented carotenoids in the two subparticles. We have shown previously [14] that two populations of carotenoids with dichroism of opposite sign and with slightly different absorption maxima were present in the intact PS II particles isolated from *Chlamydomonas*. We confirm this observation in the case of *Synechococcus* sp. (Fig. 1a) and furthermore demonstrate that CP2-c (Fig. 1c) contains the carotenoid pool absorbing at 495 and 465 nm and oriented rather perpendicular to the membrane plane, while the major carotenoid population in CP2-b (Fig. 1b) absorbs around 505 and 470 nm and is oriented close to the membrane plane. The different absorption maxima of the various carotenoid populations cannot be ascribed to a difference in the type of carotenoid molecules because highly purified PS II complexes contain only beta-carotene and no xanthophylls [35]. The linear dichroism spectrum of the intact particle in the blue and green spectral ranges is quite well described by the sum of the linear dichroism spectra of the two subparticles (data not shown). One notable exception is the 430 nm negative linear-dichroism signal in the intact particle which we thus assign to the Soret band of the reduced cytochrome *b*-559.

The 682 nm signal

In a previous study [14] we had noticed the identical wavelength position of this well-resolved shoulder in the spectra of the intact PS II particle and of the peak of the absorbance-detected magnetic resonance (ADMR) spectrum recorded at 1.2 K on the same material [36]. This ADMR signal has been assigned to the ground state of P-680, which disappears upon triplet formation. In Ref. 14 we have thus used this previous assignment, together with the known property of the in-plane orientation of the Q_Y transition of P-680 [37], to propose that the 682-nm shoulder (which is also characterized by a positive linear dichroism) in our spectra could be assigned to P-680 either alone or together with a small number of core chlorophyll *a* molecules surrounding it. The present observation (Figs. 1 and 2) that the 682 nm component of the intact particle is specifically segregated in the CP2-c photosynthetically inactive complex, while no evidence for such a component can be found in the spectra of CP2-b (Fig. 2b) is thus no longer consistent with our previous assignment [14]. Although it cannot be excluded from our data that P-680 absorbs specifically at 682 nm in CP2-b at 10 K, we will show in the following discussion that it is surprising to find no evidence for it. It has been previously demonstrated that the Q_Y transition of P-680 and of P-700 are both oriented at the same angle very close to the plane of the membrane [37]. Both in the intact thylakoids and in isolated PS I particles (Refs. 17, 20, 21 and 25, see also Breton, J., unpublished results) a positive signal is clearly visible at 697 nm in the linear dichroism spectra and corresponds to a well-defined maximum at 698 nm in the LD/A spectra. This signal can be assigned to P-700 on the basis of its large positive dichroism [21,38] and of its position which corresponds to the maximum of the absorbance changes of P-700 at low temperature [39,40]. The ADMR spectrum of PS I particles also shows a maximum at 697 nm [25] compatible with the notion that in this case the triplet is indeed localized on P-700. It is thus surprising that no special feature around the 682 nm expected position of the maximum absorption of P-680 [36] could be resolved in the linear dichroism and LD/A spectra of CP2-b. As an alternative hypothesis it can be proposed that the triplet, initially generated on P-680 by the radical-pair mechanism, migrates by triplet–triplet energy transfer to nearby chlorophyll molecule(s) absorbing at 682 nm as discussed by Rutherford [1]. If this were the case, then the conclusion that the orientation of the macrocycle(s) of P-680 is parallel to the plane of the membrane [1], which was based on measurements of the orientation of the magnetic axes of this triplet [41], should also be reinterpreted.

The 691 and 544 nm linear-dichroism signals

Clearly, the negative linear dichroism component at 691 nm (LD 691) present in the intact PS

II particle [14,20,21] is segregated in CP2-b together with the photoactive pheophytin molecule responsible for the 544 nm positive linear dichroism (LD 544) signal [14,18,20,21,27]. It has been shown previously that the Q_Y transition of this molecule is oriented rather perpendicular to the plane of the membrane [18], in the same way as are oriented the Q_Y transitions of the two bacteriopheophytin molecules in the reaction center of photosynthetic purple bacteria [29]. It has been previously demonstrated that F 695, a characteristic emission of PS II at low temperature, was specifically associated with CP2-b [7]. On the basis of this remarkable orientation (perpendicular to the membrane plane) of both the emission dipoles responsible for F 695 [15–17] and the Q_Y transition of the pheophytin acceptor [18], we have previously proposed that F 695 was emitted directly from this molecule [19]. An F 695 emission, polarized perpendicular to the plane of the particle, has been observed in isolated PS II core complexes, indicating that indeed the pheophytin acceptor could be responsible for this emission [14,20,21,42]. Furthermore, a close correlation exists between the amplitudes of F 695 and of LD 691 [14]. The maximum amplitude of this negative linear dichroism is somewhat variable around 691–693 nm depending on the origin of the particle (this study and Refs. 14, 20 and 21). In all cases the positive sign and the large magnitude of the dichroism of the other pigments absorbing in the same spectral range preclude a determination of the position of the absorption maximum of the species responsible for LD 691. Taking into account all these observations, we have further proposed that the pheophytin acceptor could be directly responsible for both LD 691 and F 695 [14,21]. The observation that LD 691 is preferentially segregated in CP2-b together with F 695 and LD 544 thus does not run contrary to our hypothesis on the origin of F 695. Furthermore, we note that the amplitude of LD 691 relative to the dichroism at the red maximum is smaller (by about a factor of 2) in CP2-b than it is in the intact particle. However, the ratio of the amplitudes of LD 691 to LD 544 is comparable in CP2-b and in the intact particle (compare Fig. 1b and a). Assuming LD 691 is due to the pheophytin acceptor as proposed in Refs. 14 and 21, this observation can be taken as an indication that a large fraction of this acceptor has been either lost or disoriented during the splitting of the intact particle into its CP2-b and CP2-c constituents. This observation goes along the report that about half of the primary charge stabilization on Q_A is lost during the isolation of CP2-b [9]. It has also been previously noticed that a large fraction of both the photoreducible pheophytin and the PS II activity was lost during the splitting of an intact particle from mesophilic organisms [5,10,11]. Furthermore, it has been reported that the isolation of CP2-b was accompanied by a significant decrease in the content of D_1 and D_2 compared to that of the core complex [9]. Our observation that only a fraction of the LD 691 signal initially present in the spectrum of the core particle (relative to the amplitude of the oriented chlorophyll *a* signal in the Q_Y region) is recovered in CP2-b can be interpreted as an indication that LD 691 is specifically associated with D_1 and D_2. Alternatively, our data cannot exclude that LD 691 is associated with the 47 kDa polypeptide of CP2-b, but that the SDS treatment utilized to isolate CP2-b from the intact particle leads to the observed partial loss of LD 691 and PS II activity.

In conclusion many of the spectral features observed in the spectra of the core particle appear remarkably well segregated in the spectra of either one of the PS II subparticles. Together with the pool of carotenoid absorbing at 495 and 465 nm and oriented in vivo mainly perpendicular to the membrane plane, the 682-nm shoulder appears in the photochemically inactive CP2-c particle, thus demonstrating that this shoulder cannot belong to P-680. On the other hand, the pool of carotenoid absorbing at 505 and 470 nm and oriented close to the membrane plane, as well as the carotenoid absorbing at 490 and 455 nm which is oriented perpendicular to the membrane, are located in the photochemically active CP2-b complex. Although LD691↑ and LD544↑ are specifically segregated in this particle, the linear dichroism spectrum of CP2-b indicates a decreased amplitude of these two spectral features compared to the spectrum of the core PS II particle. As CP2-b contains a fraction of the D_1 and D_2 polypeptides, in addition to the 47 kDa polypeptide, it cannot be concluded from the present work whether LD 691,

and thus the 695-nm emission (F 695) to which it is correlated [14], is specifically associated with the 47 kDa polypeptide or with D_1 and D_2. In order to discriminate between these two possibilities and also to gain more insight into both the structural organization and the functional role of the pigments in PS II further low temperature linear dichroism investigations are now in progress.

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