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Orientation of the pigments in Photosystem II: a low-temperature linear dichroism and polarized fluorescence emission study of chlorophyll-protein complexes isolated from *Chlamydomonas reinhardtii*

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The orientation of the various absorbing and fluorescing dipoles in Photosystem II have been investigated by linearly polarized light spectroscopy at 5 K, performed on macroscopically oriented PS II complexes derived from *Chlamydomonas reinhardtii*. Linear dichroism and absorption spectra show that the Q_Y transitions of the chlorophyll molecules are mostly tilted at less than 35° from the plane of largest cross-section of the particle (which in vivo coincides with the plane of the thylakoid membrane). The chlorophyll forms absorbing at 676 and 683 nm are oriented closer to the membrane than the forms absorbing at 665 and 670 nm which are tilted at approximately 35° from the plane. A dip observed around 680 nm in the LD / absorption spectra indicates a component tilted at a larger angle away from the membrane plane than the 676 nm- and 683 nm-absorbing species. A component weakly absorbing around 693 nm and exhibiting a negative LD (tilt larger than 35°) is clearly resolved. The amplitude of the LD at 693 nm relative to that observed at the maximum (676 nm) varies from sample to sample. In the blue spectral region, two populations of carotenoids are observed; one absorbs around 460 and 490 nm, while the other absorbs around 510 nm. They are oriented out of and near to the thylakoid plane, respectively. Comparison of polarized absorption and fluorescence spectra from the same oriented samples allows the assignment of the 695 nm fluorescence emission to the dipoles responsible for the LD signal at 693 nm.

Introduction

The photosynthetic membrane of green plants and algae is made up of a mosaic of chlorophyll-protein complexes differing in their composition and serving various functions. Some of these complexes serve only as light-harvesting antenna while others contain the Photosystem I or Photosystem II reaction centers together with a core of associated antenna pigments. The specific arrangement of the pigment molecules inside the complexes determines the high photosynthetic efficiency; thus the description of these macromolecular structures is an important step towards the understanding of the primary reactions. In the past few years there has been a wealth of procedures to isolate purified PS II particles [1–6]. However, while the composition in terms of polypeptides and prosthetic groups of these particles has received considerable atten-

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Abbreviations: PS I, II, Photosystems I, II; LHC, light-harvesting antenna complex; Chl, chlorophyll; Ph, pheophytin.

tion [7–10], there is a poverty of information concerning the structural organization of the pigments in PS II. Compared to the main light-harvesting antenna complex (LHC) or the PS I particles where circular dichroism [11], resonance Raman [12], linear dichroism [13–15] and polarized fluorescence [11,15] have been extensively investigated, there are almost no corresponding studies with the PS II particles.

In the present report we have investigated low-temperature linear dichroism and polarized fluorescence emission on the same oriented sample to characterize both the absorption and the emission properties of small PS II particles isolated from *Chlamydomonas reinhardtii*.

Materials and Methods

The mutant strain BF4:14 of the alga *C. reinhardtii* [16], which is partially deficient in the major antenna complex (LHC) and lacks the PS I reaction center, was used in this work to prepare PS II complexes as described previously [1].

Uniaxial orientation of the complexes was achieved by the polyacrylamide gel squeezing method [14]. Linear dichroism spectra were measured using linearly polarized light with the plane of polarization modulated between the vertical and horizontal directions at a frequency of 100 kHz. With synchronous detection, the difference $A_{\parallel} - A_{\perp}$ is directly obtained. Absorption is measured by recording the averaged modulated signal of the transmitted light reaching the photomultiplier. Absorption is calculated as $\log I_0/I$ (I and I_0 are the intensity of transmitted light with and without sample). Polarized fluorescence emission spectra were recorded at 77 K on the same samples oriented in squeezed polyacrylamide gels [15]. Excitation light at 640 nm was provided by a dye laser – Argon laser combination and a double monochromator was used to analyse fluorescence spectra. Spectra $A_{\parallel}$, $A_{\perp}$, $F_{\parallel}$, $F_{\perp}$ refer to the absorption and fluorescence of light polarized parallel and perpendicular to the long axis of the oriented objects [14]. Spectra were digitized on a Tracor Northern 1710. The absorption and LD spectra presented here are normalized to a value of one for the amplitude at the red maximum. A variable temperature cryostat (SMC, France) using a flow of helium gas was adjusted for low-temperature measurements.

Results and Discussion

Orientation of absorption dipoles

The absorption, LD and LD/absorption spectra at 5 K of the PS II particles isolated from *C. reinhardtii* BF4:14 and oriented in a squeezed polyacrylamide gel are presented in Fig. 1a, b and c, respectively, for the spectral range 380–750 nm. In Fig. 2a–c only the expanded spectra in the region 630–710 nm are shown. Apart from the improved spectral resolution, the spectra depicted in Fig. 1a and b are very similar to the ones reported for the same particles at 100 K [17]. As previously noted [17,18], these spectra are also very similar to the ones obtained for the other highly purified PS II particles isolated from either *C. reinhardtii* 54:14 [1,16] or from higher plants [9,19]. The main difference observed between the

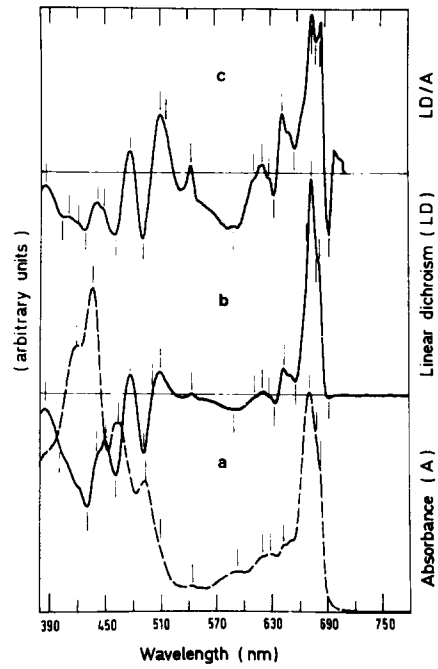

Fig. 1. a, absorption (A); b, linear dichroism (LD); c, LD/A ratio of PS II complexes derived from *C. reinhardtii* (BF4:14). Spectra were recorded between 380 and 780 nm at 5 K on complexes oriented in squeezed polyacrylamide gels. Spectra are normalized to an arbitrary value; the maximum dichroic ratio is 1.3 at 676 nm.

particles isolated from *C. reinhardtii* and from higher plants appears to be the presence of a 654 nm band in the spectra of the former. This band might correspond to the minor chlorophyll (Chl) *a* form absorbing at this wavelength which is present in various algae but not in the higher plants [20].

In the spectral range 630–710 nm, where the Q_Y transitions of Chl *a* are involved, the LD is mostly positive (Fig. 2b) indicating that the overall orientation of most of the Q_Y transition dipoles is tilted at more than 55° from the direction of applied pressure in the gel. It has been previously shown [18] that in the gel these complexes behave like flattened ellipsoids in which the pigments exhibit an orientation identical to the one they have in the native thylakoid membrane. The PS II particles have a rod-like appearance by electron microscopy [1] with a short axis of 8 nm and a length of 10–20 nm. These objects probably represent linear aggregates which include a variable number of individual reaction centers, each having a length of 8 nm along the transmembrane axis. When such objects are included in the polyacrylamide gel matrix, further aggregation induced by hydrophobic interactions could lead to larger particles behaving like flattened ellipsoids. 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 largest cross-section of the flattened ellipsoid, in the case of isolated particles, or the plane of the membrane, in the case of the intact thylakoid.

In the absorption spectrum (Fig. 2a), the maximum is located at 670 nm with two shoulders at approximately 680 nm and at 683 nm, while in the

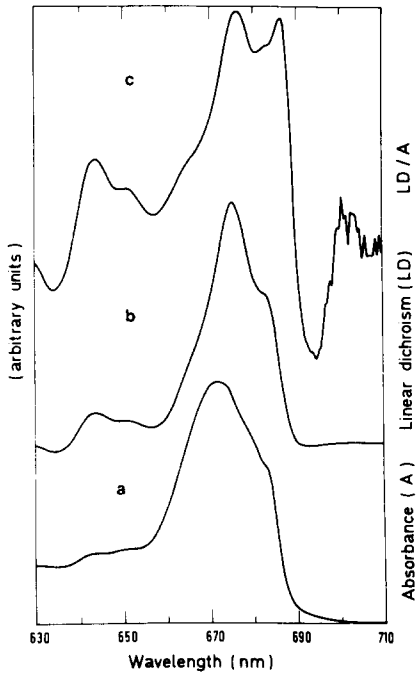

Fig. 2. The same spectra as in Fig. 1, amplified in the region 630–710 nm.

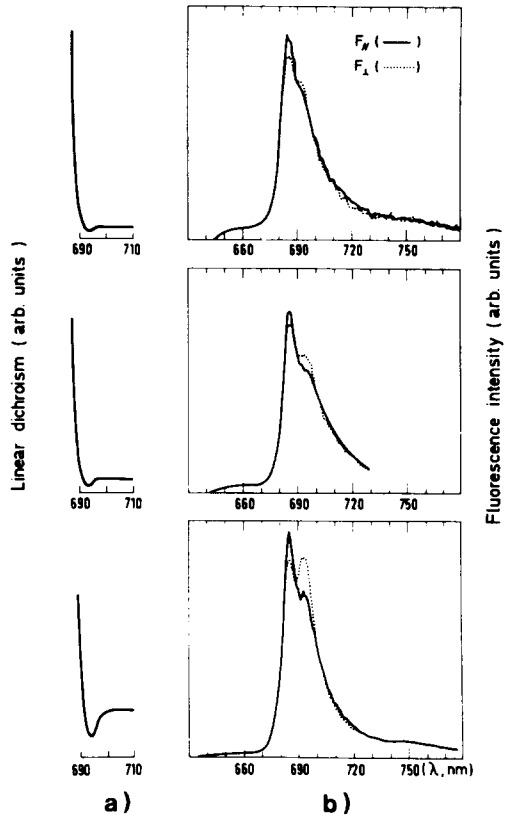

Fig. 3. (a) Long-wavelength side of the LD spectra. The three spectra have been normalized to an equal value at the LD peak. Spectra were recorded at 100 K from three different samples of PS II complexes derived from *C. reinhardtii* BF4:14. (b) Polarized fluorescence emission spectra at 77 K of the complexes presented in (a), obtained in the same squeezed gels. $F_{\parallel}$ (———) and $F_{\perp}$ (·····) are recorded parallel and orthogonal to the largest axis of the complexes, respectively. The bottom spectra are recorded from the sample whose LD, absorption (A) and LD/A spectra at 5 K are shown in Figs. 1 and 2.

LD spectrum (Fig. 2b) the maximum is located at 676 nm (dichroic ratio, 1.3) with a prominent shoulder at 683 nm (and a small feature at approx. 680 nm). In addition, a small trough is observed at 693 nm in the LD spectrum which leads to a distinct negative band in the LD/absorption spectrum (Fig. 2c). As shown in Fig. 3a, the amplitude of this trough relative to the 676 nm LD maximum varies largely from sample to sample, so that in some cases (Fig. 3a, top) it is just barely visible in the 100 K LD spectra. However, in such cases the LD/absorption spectra still clearly indicate the presence of a component with negative LD at 693 nm and corresponding to a transition dipole tilted at less than 55° from the normal to the plane of the particle.

Around 660–680 nm the absorption and LD spectra can be explained by the presence of three transitions at 665 nm (large absorption, small positive LD), at 670 nm (medium absorption, no LD) and at 676 nm (large absorption and large positive LD). Some of these transitions have been already postulated to explain low-temperature absorption and fourth-derivative spectra of PS II particles [4,21,22]. We also note that the presence of a Chl *a* form absorbing at approximately 670 nm and with its Q_Y dipole oriented at 35° from the membrane plane (no LD) has been already reported in thylakoids [23], as well as in LHC and PS I [14,15].

The amplitude of the 683 nm shoulder (Fig. 2a,b) relative to the whole absorption band allows us to estimate that two to six Chl *a* molecules contribute to this component. The LD/absorption value is close to that observed for the 676 nm band and indicates that the corresponding dipoles are oriented at less than 30° from the plane of the particle. It has been reported that the long-wavelength transition of the primary donor of PS II (P-680) is oriented at approximately 25° from the membrane plane [24]. Furthermore, the triplet-minus-singlet spectrum measured at 2 K on the particle isolated from the 54-14 mutant indicates an absorption maximum of 682.5 nm for P-680 [25]. It is thus possible to ascribe to P-680 at least part of the 683 nm component resolved in the absorption and LD spectra at 5 K. The remaining portion of the 683 nm component could correspond to a small core of antenna pigments located in the vicinity of P-680 and exhibiting an orientation of their Q_Y transition dipole similar to that of P-680. In this respect, it should also be noticed that components absorbing close to 683 nm have been reported in the 77 K absorption spectra of the non-photochemically active PS II particles CP IV [3] and CP2c [5].

The 680 nm shoulder observed in the absorption spectra (Fig. 2a) has no counterpart in the LD spectra (Fig. 2b). Instead, a slight depression is usually discernible in the LD and LD/absorption spectra. This indicates that this 680 nm component exhibits either no LD or a negative LD. It is known that the intermediary electron acceptor of the PS II is a pheophytin (Ph) molecule [26]. The photochemical reduction of this acceptor is accompanied by a bleaching centered close to 685 nm at room temperature. LD investigation of this bleaching [27] has shown that the Q_Y transition of this Ph, which absorbs around 680 nm at room temperature, is oriented at a large angle out of the plane of the particle. It is possible that the small dip observed in the LD/absorption spectra around 680 nm corresponds to this species. The 693 nm negative LD could also correspond to the long-wavelength wing of the absorption band of Ph if the wavelength of the maximum shifts to the red upon lowering the temperature. In this case the 680 nm shoulder in the absorption spectrum should be assigned to some other pigment(s). It is clear that the opposite sign of the LD signals arising from the 683 and 693 nm components makes difficult the precise determination of the absorption maximum of the minor 693 nm component.

In the spectral range 630–530 nm (Fig. 1) two broad absorption bands are observed around 620 and 585 nm. The 620 nm band, with which a three-component LD signal is associated, probably corresponds to vibrational $Q_{Y0\text{-}1}$ transitions. We tentatively ascribe the negative LD signal associated with the 585 nm band to Q_X transitions of Chl *a* tilted at more than 35° out of the plane of the particle. At 543 nm a sharp positive LD signal is associated with a small absorption band assigned to a transition of the Ph molecules [27,28]. The amplitude of this band is compatible with the presence of two Ph per approx. 50 chlorophylls [29]. In the LD/absorption spectrum (Fig. 1c) the presence of this spectral component is very promi-

nent, in contrast to its absence from the LD/absorption spectrum of PS I [15] and LHC (Tapie, P. and Breton, J., unpublished data).

In the spectral range 530–450 nm, the absence of Chl *b* in the PS II particles utilized in this study (P. Delepelaire, personal communication) allows the observation of two populations of carotenoids with dichroism of opposite signs. The largest fraction oriented at more than 35° out of the membrane plane exhibits LD peaks at 490 and 460 nm. The smaller fraction, oriented closer to the membrane plane, is responsible for the positive LD peak at 510 nm. Two functionally different pools of carotenoids have been recognized in PS II; the first one is quencher of the triplet state of P-680 [30], while the second one can act as an accessory electron donor to P-680 [31]. Thus it is possible that the two populations of carotenoids exhibiting different orientations have different functional roles.

Orientation of the emission dipoles

The polarized emission spectra at 77 K of the same oriented specimen utilized to record the absorption (Fig. 1a) and LD (Fig. 1b) spectra are presented in Fig. 3b (bottom). Two maxima at 685 nm ($F_{\parallel}/F_{\perp} = 1.15$) and at 695 nm ($F_{\parallel}/F_{\perp} = 0.85$) can be seen. We have observed that the amplitude of the F-695 emission relative to F-685 is quite variable from sample to sample, as shown by the three pairs of emission spectra depicted in Fig. 3b. A large variability in the ratio F-695/F-685 of isolated particles either from batch to batch, or for various isolation procedures, or upon various treatments has been reported [8,10,32–34]. We note that in all cases where a F-695 emission was present in our preparations, its anisotropy ratio $F_{\parallel}/F_{\perp}$ was always smaller than 1.

In intact chloroplasts the 77 K fluorescence emission is composed of three main bands at 685 nm (F-685), 695 nm (F-695) and 735 nm (F-735) with a small shoulder at 680 nm (F-680). While the PS I origin of F-735 is well accepted, there is still some controversy over the origin of the other bands. F-685, which had for a long time been ascribed to LHC [35], was later assigned to PS II [36] and it has been shown that F-680 originates from LHC [37,38]. The fluorescence emission at 695 nm has been attributed to P-680 [39], to energy-trapping centers appearing in the PS II antenna at temperatures below 100 K [35,40], to an aggregated state of LHC [41] or to the Ph intermediary acceptor of PS II [42]. Polarized fluorescence measurements on oriented chloroplasts [38,43,44] have shown that F-695 is emitted from pigments preferentially oriented at a large angle away from the membrane plane. This observation has been used to demonstrate that F-695 can originate neither from P-680 [43] nor from LHC [45,46]. On the other hand, the orientation of F-695 emission dipoles close to that of the Q_Y transition of the Ph intermediary acceptor of PS II, led to the proposal that the F-695 emission stems from this species [42]. This model has received some support from the observation of a specific quenching of F-695 upon photoreduction of the intermediary acceptor [47,48].

The polarized emission spectra depicted in Fig. 2b clearly show that F-695 originates in PS II and is emitted from dipoles tilted at more than 35° out of the plane of the PS II particle. The LD/absorption spectrum (Fig. 2c) indicates that the dipoles responsible for the 693 nm component (and maybe the 680 nm component) exhibit an orientation that is qualitatively comparable to that of the dipoles emitting F-695. Furthermore, by comparing the low-temperature LD and fluorescence emission spectra of a variety of PS II complexes isolated from *C. reinhardtii* BF4:14 (Fig. 3), as well as from differents mutants of *C. reinhardtii* and from higher plants (data not shown), the amplitude of F-695 (relative to F-685) appears qualitatively well correlated with the amplitude of the negative LD signals at 693 nm (LD 693) relative to the LD peaks at 676 nm, as shown in Fig. 3a and b. This demonstrates that the 695 nm fluorescence and the LD 693 signal probably have a similar origin. Two hypotheses can be considered regarding the molecular origin of the species responsible for the LD signal at 693 nm; it could arise either from a single long-wavelength absorbing pigment with no photochemical activity or from the photochemically active Ph.

On the basis of polarized fluorescence excitation spectra of oriented chloroplasts, F-685 has been ascribed to a Chl *a* absorbing at 676 nm (C676) because both dipoles have a similar orientation with respect to the membrane plane [38]. However, this conclusion is ambiguous, as efficient energy transfer from C676 of LHC to PS II

can mask the real origin of the dipoles emitting F-685. The measurements on isolated PS II particles reported here do not allow us to discriminate which of the 676 or the 683 nm components is responsible for F-685, because they have similar LD/absorption values. Furthermore, the dichroic ratio of C-676 is higher than the anisotropy ratio of F-685 (Fig. 3), indicating that F-685 is probably contaminated by some disconnected pigments. Finally, it is possible that both C676 and C683 of PS II contribute to F-685 as suggested previously [49].

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