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Spectroscopic properties of chloroplast grana membranes and of the core of Photosystem II

R.J. van Dorssen, J.J. Plijter, J.P. Dekker, A. den Ouden, J. Amesz
and H.J. van Gorkom

Department of Biophysics, Huygens Laboratory, State University of Leiden, P.O. Box 9504,
2300 RA Leiden (The Netherlands)

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An oxygen-evolving Photosystem II core complex essentially free of the light-harvesting chlorophyll *a/b* protein complex, containing 45 chlorophylls per reaction center was isolated from spinach chloroplasts. Its structural integrity was established by studying its photochemistry and spectral properties. The absorption spectrum measured at 4 K revealed the presence of at least five spectrally distinct chlorophyll *a* species. The same bands, but in different proportions, were observed in a Photosystem II grana preparation used as starting material for the preparation of the core complex. The relative contributions of these components to the overall absorption were calculated by deconvoluting this spectrum into Gaussian bands. The core complex was enriched in a long-wave band located at 683 nm, which presumably reflects the presence of 8–10 pigment molecules that are closely associated with the reaction center. Low temperature fluorescence emission spectra showed the characteristic Photosystem II emission bands located at 685 nm (F_{685}) and at 695 nm (F_{685}). The two states giving rise to these emissions are in thermal equilibrium down to 70 K. It is suggested that F_{685} arises from a chlorophyll *a* species absorbing at 676 nm and that F_{695} is the result of fluorescence from the photoactive pheophytin *a* absorbing around 683 nm.

Introduction

Photosystem II of green plant photosynthesis consists of a complex of a large number of peptides, which are embedded in the thylakoid membrane. A considerable fraction of the total pigment and protein content of PS II is made up by the light-harvesting Chl *a/b* protein complex (LHCP) [1]. The remaining part, which we shall call the core complex, consists of an aggregate of at least five different intrinsic proteins [2,3] to which several extrinsic proteins are bound that are involved in oxygen evolution [4]. Most of these proteins appear to be colorless and have a function in electron transport only, but some of the peptides of the core complex contain considerable amounts of Chl *a*, which acts as light-harvesting pigment to absorb and transfer energy to the reaction center.

A method for the isolation of a purified grana membrane sheet preparation that contains almost pure PS II has been described by Berthold et al. [5], and more recently it has been shown that this

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Abbreviations: CD, circular dichroism; Chl, chlorophyll; PS II, Photosystem II; LD, linear dichroism; LHCP, light-harvesting chlorophyll *a/b* protein; Mes, 4-morpholineethanesulfonic acid; Pheo, pheophytin; RC, reaction center.

Correspondence: J. Amesz, Vakgroep Biofysica, Huygens Laboratorium, Rijksuniversiteit Leiden, postbus 9504, 2300 RA Leiden, The Netherlands.

preparation can serve as starting material for the preparation of PS II core complexes of various degrees of purity [6–8]. These core complexes are quite useful material to study the structural and photochemical properties of PS II.

The present communication describes the photochemical and spectroscopic properties of a purified oxygen-evolving core complex, in comparison with those of a purified PS II grana preparation and of intact chloroplasts. Measurements of absorption, fluorescence and circular and linear dichroism indicate that the structural integrity of these preparations is retained during the isolation procedure. Evidence concerning the orientation and assignment of the various absorption and fluorescence transition dipoles in PS II will be presented.

Materials and Methods

PS II complexes were prepared from spinach chloroplasts according to Berthold et al. [5], except that the second Triton X-100 incubation was omitted. The final preparation (which will be called the BBY complex after the authors' names of Ref. 5) was stored in 400 mM sucrose, 50 mM Mes, 15 mM NaCl and 5 mM $MgCl_2$ (pH = 6.0) in liquid nitrogen at a Chl concentration of 3 mg/ml. The BBY complex was used as starting material for the preparation of an oxygen-evolving PS II core complex by solubilization with 1-*O*-*n*-octyl-β-D-glucopyranoside (octylglucoside) by an adaptation of the method described by Ikeuchi et al. [6]. A considerable increase in purity was obtained by improved sucrose gradient centrifugation in the presence of 60 mM detergent with a linear gradient (27–35%, w/v) on top of a 55% sucrose layer. The gradient centrifugation was carried out in a Beckman Ti70 rotor at 175000 × *g* for 16 h. Oxygen evolution was measured with a Clark-type O_2 electrode at 23°C in a buffer containing 50 mM Mes/15 mM NaCl/5 mM $MgCl_2$ (pH = 6.0). Chl *a* and *b* concentrations and ratios were determined according to Arnon [9]. The concentration of the secondary acceptor Q_A was measured by the light-induced absorbance increase at 325 nm [10] in the presence of 2.5 mM ferricyanide and 10 μM 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out on 1.25% acrylamide gels according to Laemmli [11]. The apparatus used to measure absorption and fluorescence emission and excitation spectra and fluorescence polarization is described in Ref. 12. Linear and circular dichroism spectra were recorded with the apparatus described in Ref. 13. For measurement of LD the samples were oriented uniaxially or biaxially by pressing a polyacrylamide gel as described in Ref. 14.

Results

Composition and photochemical activity

Upon sucrose gradient centrifugtion of the detergent-treated BBY complex (see Materials and Methods), seven colored bands were formed. Most of the green material settled on the top. This band was found to consist mainly of the light-harvesting chlorophyll *a*/*b* protein complex (LHCP) on basis of its absorption spectrum and its low Chl *a*/*b* ratio, while six green bands were formed below (Table I). The band on top of the 55% sucrose layer (band 7) consisted of the PS II core complex. It had a Chl/RC ratio of 45 as determined by the amount of photoreducible Q_A, and was practically devoid of Chl *b*. Gel electrophoresis showed that the core complex contained the 47, 43, 34–33 (D2 and the recently described protein [6]), 32 (D1) and 10 kDa intrinsic and the 33 kDa extrinsic

TABLE I

PROPERTIES OF THE BBY COMPLEX AND OF THE FRACTIONS IN THE SUCROSE GRADIENT

Fraction	Chl/Q_A	Chl *a*/*b*	O_2 evolution [a]	Yield of recovery (%)
BBY	260	2.15	290 [b] 45 [c]	
Band 1	> 5000	1.45	n.d.	75
Band 2	2500	6.5	n.d.	6
Band 3	270	7.5	n.d.	3
Band 4	170	13.5	n.d.	2
Band 5	100	> 15	n.d.	1
Band 6	65	> 20	n.d. 80 [c]	2
Band 7	45	> 20	80 [b] 150 [c]	4

[a] Expressed in μmol per mg Chl per h.

[b] 2,5-Dichloro-*p*-benzoquinone (300 μM) used as electron acceptor.

[c] Ferricyanide (2.5 mM) used as electron acceptor.

proteins (see Ref. 2) in approximately equal molar ratios, while a significant fraction of the extrinsic 24 kDa protein was also retained.

Rates of oxygen evolution were measured in saturating light with either ferricyanide or 2.5-dichloro-*p*-benzoquinone as electron acceptor. With the BBY complex the highest rate was obtained with 2,5-dichloro-*p*-benzoquinone [15]. The opposite was observed with the core complex, where the highest activity was obtained with ferricyanide. This indicates that the low rates of oxygen evolution exhibited by this preparation are due to a rate-limiting step on the reducing rather than on the oxidizing side of PS II, resulting in a low rate of electron transfer especially with a lipophilic acceptor. This is also indicated by the fact that no stimulation of the oxygen evolution by $CaCl_2$ could be found in the core complex, in contradiction to the results obtained by Ikeuchi et al. [6] and Ghanotakis et al. [7] with their preparations. Bands 6 to 2 (in this order) showed a decreasing reaction center activity and a decreasing Chl *a/b* ratio. The peptide content, and except for band 6, the oxygen-evolving capacity of these fractions were not investigated. They may have consisted of associations of the core complex and LHCP, but the high Chl/Q_A ratio indicates a partial loss of activity in these fractions.

Absorption spectra

Low-temperature absorption spectra of the BBY complex and of the core complex are shown in Figs. 1 and 2. The most conspicuous differences between the two spectra are the strongly reduced Chl *b* content in the core complex as reflected by the absence of the bands near 650 and at 472 nm and the much higher proportion of long-wave absorbing pigment as indicated by the shoulder near 683 nm. In the spectrum of the core complex carotenoid bands are visible at 505 and 467 nm, which are probably due to β-carotene [16] and which are partly obscured in the spectrum of the BBY-complex; the spectrum of the latter preparation shows additional peaks and shoulders in the carotenoid region that are presumably due to the Chl *a/b* light-harvesting complex.

In the Q_y region the spectra are composed of several overlapping absorption bands. The approximate peak wavelengths of these bands, obtained from the second derivatives of the spectra, are listed in Table II. It can be seen that the number and positions of the bands agree quite well with those obtained by Kramer et al. [17] for spinach chloroplasts from excitation spectra of photosystem II fluorescence. This indicates that the structure of the complexes was left virtually intact by the isolation procedure.

In order to determine the relative contributions

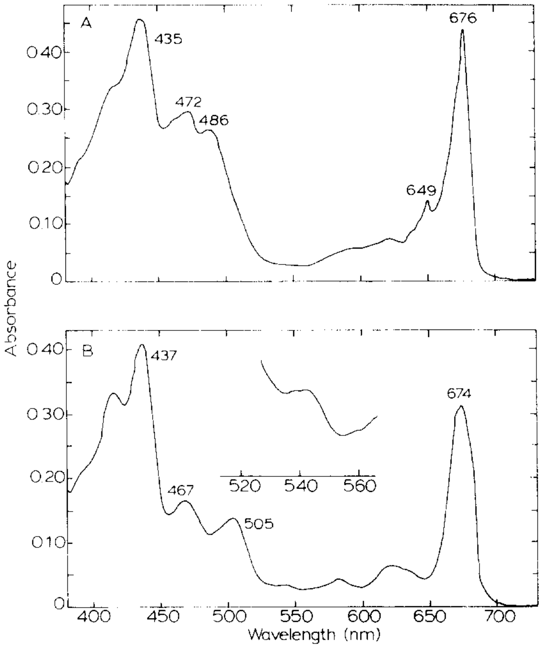

Fig. 1. Absorbance spectra of the BBY complex (A) and of the core complex (B) at 4 K. The inset of Fig. 1B shows the region between 530 and 560 nm on a 3-fold expanded scale.

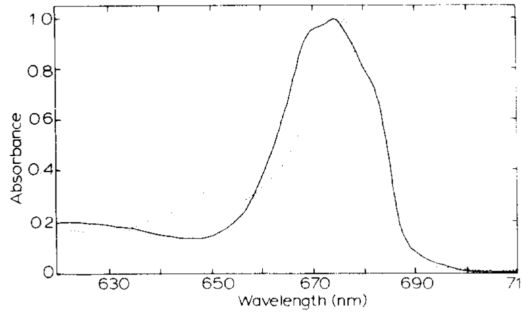

Fig. 2. Absorbance spectra of the Q_y region of the BBY complex (· · · · · ·) and of the core complex (———) at 4 K.

TABLE II

GAUSSIAN DECONVOLUTION OF THE ABSORPTION SPECTRA OF THE BBY COMPLEX AND THE CORE COMPLEX IN THE Q_y REGION AT 4 K

The relative intensities of the bands were obtained by plotting A/ν vs. ν (where ν is the frequency) and integrating this expression over the band. They are related to the total dipole strength of all bands. In the case of the core complex the 649 component is excluded because of its doubtful origin.

Band center (nm)		Half-width (nm)		Amplitude		Relative intensity	
BBY	core	BBY	core	BBY	core	BBY	core
649	649	11	10	0.13	0.04	18.9	–
659	660	9	10	0.11	0.08	12.9	12.8
669	668	9	10	0.22	0.21	26.8	33.5
676	675	8	9	0.36	0.23	37.9	34.0
684	682	7	8	0.04	0.17	3.4	19.7

of the various Chl *a* and *b* bands to the overall absorption spectra, the spectra were deconvoluted in Gaussian components [18], using the peak positions determined from the second derivative spectra. For both preparations a reasonable fit could be obtained for the region 640–690 nm. The halfwidths of the corresponding bands were approximately the same for the two spectra. The amplitudes and the relative intensities are listed in Table II.

The main differences observed are the much higher proportion of long-wave absorbing pigment (683 nm) in the core complex and the decreased proportion of the component absorbing near 649 nm. In the BBY complex this band is mainly due to Chl *b*. For the core complex the band is of doubtful physical importance, and may reflect a deviation from the Gaussian shape of the other bands, since it coincides with a minimum in the absorption spectrum and was not observed in the second derivative. The band at 683 nm is enhanced about 5-fold in the core as compared with the BBY complex. Approximately the same increase was observed in the reaction center content, as measured by the light-induced absorbance increase at 325 nm due to Q_A reduction. This indicates that all of the 683 nm band in the BBY complex belongs to the core complex, and may be associated with the reaction center. Part of the absorption band may in fact be accounted for by P-680 and Pheo *a* [19–21], the remaining being due to Chl *a* (Chl a_{683}). Our analysis indicates that 20% of the total Chl $(a+b)$ present in the BBY complex belongs to the core complex, the remaining being contained in the LHCP. A minor component near 693 nm accounts for the long-wave 'tail' in the region above about 685 nm in the spectra of the BBY and core complexes (see Fig. 2). The possible origin of this component, which is enriched in the core complex, will be discussed below. The band near 540 nm which has been attributed to Pheo [8] upon closer inspection (Fig. 1B, inset) appears to be composed of two components, with maxima at 537 and 543 nm as indicated by the second derivative spectrum.

Fluorescence

Fluorescence emission spectra of the two preparations upon excitation at 440 nm in the Soret region of Chl *a* were measured at various temperatures. At room temperature most emission occurred in a band located at 685 nm (F_{685}) for both complexes (Fig. 3). Below 150 K a second emission band around 695 nm (F_{695}) appeared. The intensity of F_{695} increased upon further cooling and at 4 K practically all emission occurred from this band. Emission at wavelengths above 700 nm was relatively weak, as was earlier concluded for Photosystem II emission from whole chloroplasts [22]. The emission band at 754 nm is probably a vibrational sub-band of the main emission at 695 nm in agreement with earlier conclusions from excitation spectra [12]. At 4 K both preparations also showed minor bands at 715 and 730 nm.

Fig. 4 shows the temperature dependence of the relative intensities of the emission bands at 685

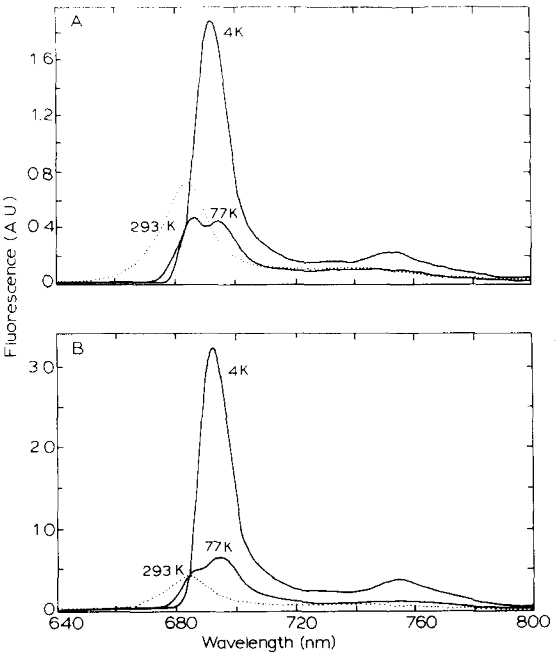

Fig. 3. Emission spectra of the BBY complex (A) and of the core complex (B) excited at 440 nm and measured at various temperatures.

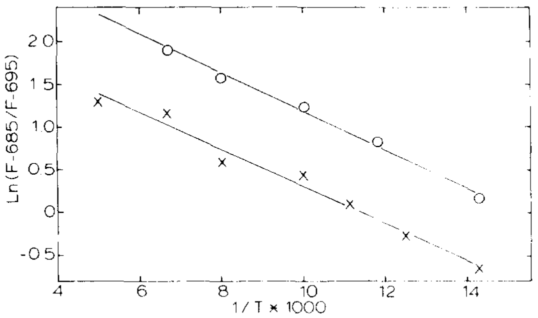

Fig. 4. Temperature dependence of the ratio of the F_{685} and F_{695} emission yields. Excitation at 440 nm. The spectra were deconvoluted as described in the text. × ——— ×, core complex; ○ ——— ○, BBY complex.

and 695 nm obtained by deconvolution of the emission spectra obtained at various temperatures. Semi-logarithmic plots of the ratios of the intensities vs. the reciprocal of the temperature yielded straight lines for both preparations down to 70 K, indicating that a thermal equilibrium exists between the two emissions. This equilibrium can be described by a Boltzmann equation:

$$\frac{\phi_{685}}{\phi_{695}} = \frac{N_{685}}{N_{695}} \frac{K_{685}}{K_{695}} e^{-\Delta E/kT} \qquad (1)$$

where N_{685}/N_{695} is the ratio of the number of molecules giving rise to the emissions at the two wavelengths, and K_{685}/K_{695} is the ratio of their yields of fluorescence. The slope of both lines yields an energy difference $\Delta E = 155 \pm 5$ cm^{-1} corresponding to a difference in the absorption maxima of the two states of approx. 7 nm. The intercepts with the *y*-axis give the value for $(N_{685}/N_{695})(K_{685}/K_{695})$, resulting in 31 for the BBY complex and 12 for the core complex.

For both complexes the emission spectra indicate that there must be efficient energy transfer to the long-wavelength pigments. This is confirmed by the fluorescence excitation spectra of the 695 nm emission shown in Fig. 5. Comparison of these spectra with the absorption $(1 - T)$ spectra showed an efficiency of energy transfer exceeding 90% at all wavelengths, except for those absorbed by carotenoids. The amplitudes of the 502 and 467 nm bands in the excitation spectrum of the core complex indicated an efficiency of energy transfer

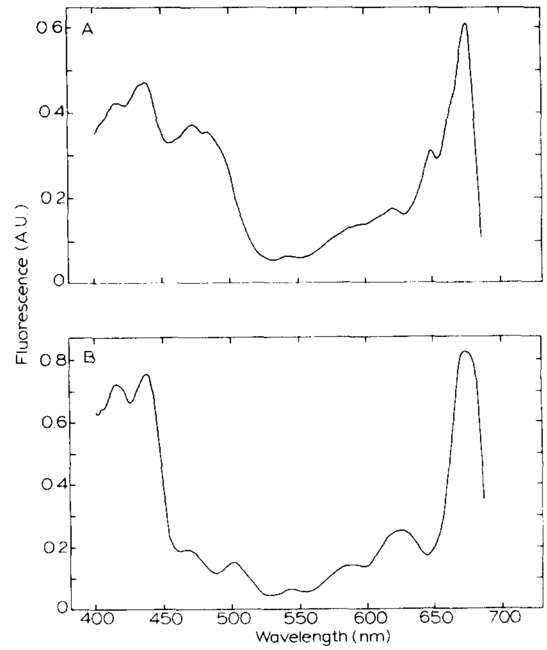

Fig. 5. Fluorescence excitation spectra of the BBY complex (A) and the core complex (B) at 4 K. Detection wavelength, 696 nm. The absorbances of the samples were the same as in Fig. 1.

from carotenoid to Chl *a* of approx. 35%. This spectrum is similar to that for PS II emission [17] from an LHCP-less mutant of barley, except that the carotenoid bands are located at somewhat longer wavelengths. For the BBY complex the efficiency of energy transfer from carotenoid to Chl appears to be much higher. This energy transfer occurs apparently mainly in LHCP. Very similar excitation spectra were obtained for the 755 nm emission (not shown).

Fig. 6 shows the fluorescence polarization spectra for excitation of the 695-nm emission in both complexes. The polarization value p defined as $p = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$ was approx. 0.10 for most of the Q_y region but rose steeply above 680 nm. This either indicates an approximately parallel alignment for the long-wave emitting dipoles or the absence of energy transfer between them.

Linear dichroism

Both preparations were oriented uniaxially by pressing a polyacrylamide gel between two parallel prisms as described in Ref. 14 from an initial thickness of 5 mm to a final one of 2 mm. The measuring beam passed the sample at an angle of 50°. As the Q_y transitions of Chl *a* are oriented mainly parallel to the plane of the thylakoid membrane [12,23,24], the positive LD signal from the BBY complex (Fig. 7A) in this region indicates that upon squeezing the particles are aligned with their membrane sheets parallel to the face of the prisms.

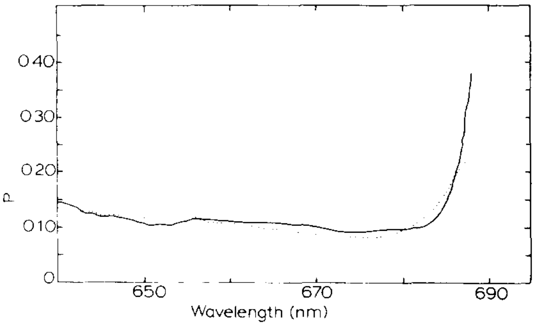

Fig. 6. Fluorescence polarization spectra of the BBY complex (· · · · ·) and of the core complex (———) at 4 K. Detection wavelength, 696 nm.

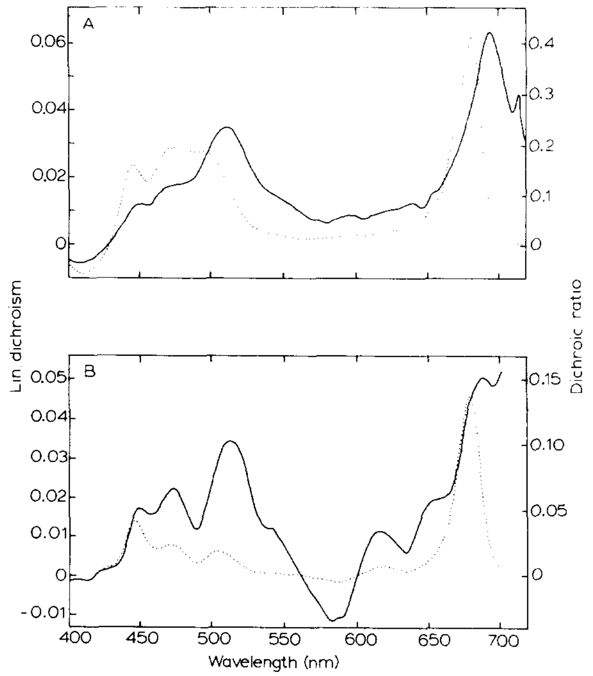

Fig. 7. Linear dichroism spectra, $A_{\parallel} - A_{\perp}$ (· · · · · ·) of the BBY complex (A) and of the core complex (B) oriented by uniaxial pressing at 293 K. The solid lines indicate the dichroic ratio. Absorbances in the Q_y maxima were 0.24 and 0.37, respectively.

The dichroic ratio, defined as $(A_{\parallel} - A_{\perp})/(A_{\parallel} + A_{\perp})$, of the BBY complex rises steeply across the Q_y band region and reaches a maximum at about 690 nm. This indicates that the Q_y transitions of the long-wave chlorophylls, presumably Chl a_{676} and Chl a_{683} are oriented more parallel to the membrane than those absorbing at shorter wavelengths, in general agreement with earlier observations with chloroplasts [23]. The large dichroic ratio near 690 nm indicates a high degree of orientation of the membranes. A linear relationship between the dichroic ratio and the degree of pressing was observed at this wavelength, up to the maximum degree of pressing applied, where a dichroic ratio of 0.5 was obtained. This suggests that the 'true' dichroic ratio is at least 0.6, as compared to the maximum value of 0.90 for our geometry, corresponding to an angle of at least 70° with the normal to the plane of the membrane. If we take into consideration that one of the pigments that contribute to the measured dichroic ratio is probably Pheo *a*, the Q_y transition of which makes a small angle to the normal of the membrane [20], the orientation angle for Chl *a* 683 is probably between 80° and 90°. The spectrum further shows the characteristic Chl *b* signal [12,23] near 650 nm and a positive signal for the carotenoid region. The LD spectrum of the core complex (Fig. 7B) shows roughly the same features as the spectrum of the BBY complex, but the lower dichroic ratio suggests that much less orientation was obtained with this complex. In the carotenoid region the dichroic ratio varies with wavelength with maxima at 514 and 474 nm and minima at 488 and 459 nm. This indicates the presence of two pools of carotenoid as earlier observed [24].

The LD spectrum of the BBY complex oriented biaxially [14] and measured at 4 K is shown in Fig. 8. With this method the membranes are aligned preferentially parallel to the stretching axis of the gel, as is confirmed by the positive LD in the Q_y region of the Chl *a*. Although both the LD and absorption spectra have their maxima at 676 nm, there are some major differences between these two spectra. Both the 670 and 662 nm bands are much less pronounced in LD, whereas Chl a_{676} and Chl a_{683} give a strong contribution to the LD spectrum. This is also reflected by the dichroic ratio, which has its maximum at 682 nm. An additional maximum can be seen at 686 nm and a minimum at 695 nm, while the minimum at 649 nm indicates that the Chl *b* transitions are tilted at more than 35° out of the plane of the membrane. In the visible region, the Pheo *a* band at 543 nm shows up clearly in the dichroic ratio spectrum and its transition dipole appears to make a small angle with the plane of the membrane. The dichroic ratio in the carotenoid region again gives evidence for two pools of carotenoid.

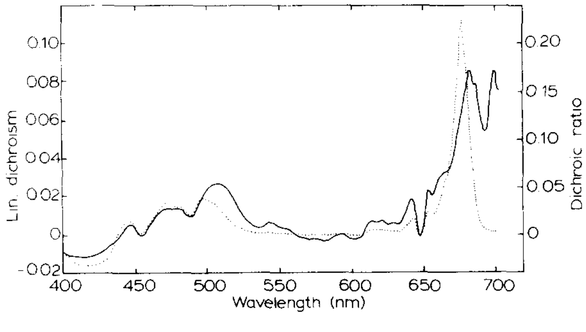

Fig. 8. Linear dichroism spectrum (······) of the BBY complex oriented by biaxial pressing at 4 K. The solid line indicates the dichroic ratio. The absorbance in the Q_y maximum was 0.86.

Even at the maximum at 682 nm, the dichroic ratio of 0.17 is only small as compared with the theoretical maximum value of 3.0 [14], which indicates a low degree of orientation. From the uniaxially oriented BBY complex it was concluded that the 683 nm transitions probably make an average angle of at least 80° with the normal to the plane of the membrane. This in turn allows an extrapolation of the data from the biaxially oriented BBY complex to a situation of perfect orientation. From the thus obtained 'true' dichroic ratio the orientations with respect to the normal of the thylakoid membrane can be calculated for the different Chl *a* Q_y transitions, resulting in angles of $60 \pm 1°$, $63 \pm 1°$ and $70 \pm 2°$ for Chl a_{660}, Chl a_{670} and Chl a_{676}, respectively.

We also measured the polarized emission spectra of the biaxially oriented BBY complex. The spectra (not shown) were recorded at 70 K, at which temperature the intensities of F_{685} and F_{695} are roughly equal. The ratio $F_{\|}/F_{\perp}$ had a maximum at 685 nm and a minimum at 695 nm, in agreement with measurements of Tapie et al. [24] on PS II preparations from the green alga *Chlamydomonas reinhardtii*. This indicates that the pigments emitting F_{685} are oriented preferentially parallel to the plane of the membrane, whereas F_{695} is emitted by pigments making a large angle with this plane.

Circular dichroism

The CD spectra of both preparations measured at room temperature and at 77 K are shown in Fig. 9. Apart from a higher resolution the 77 K spectra closely resemble those measured at room temperature. The spectra of the BBY complex are dominated by the signal of LHCP [25] with characteristic bands at 642, 654, 670 and 685 nm. This spectrum has been explained in terms of a trimeric exciton model [26], where two Chl *b* exciton states give rise to the peak at 654 nm, whereas the signal at 670 nm results from the third exciton state. The splitting of the negative band around 650 nm, which is much more pronounced at 77 than at 300 K, has been ascribed to interaction between Chl *a* and Chl *b* [27]. The blue region of the BBY

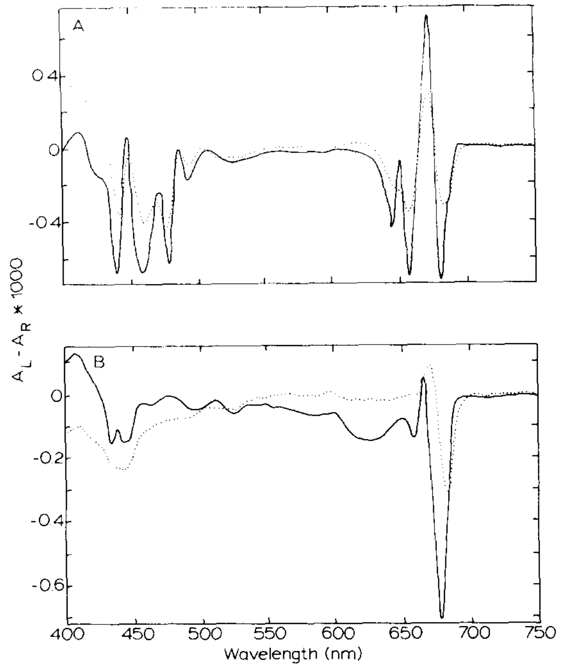

Fig. 9. Circular dichroism spectra of the BBY complex (A) and of the core complex (B) measured at 293 K (······) and at 77 K (———). Absorbances at the Q_y maxima at 293 K were 1.0 and 0.27, respectively.

spectrum shows, apart from the contributions of Chl *a* at 440 nm and Chl *b* at 478 nm, bands of carotenoid at 460, 483, 491 and 504 nm. The spectrum of the core complex shows a strong negative band at 679 nm, which is also present in the BBY complex and weaker bands can be seen at 659 and 665 nm. Relatively small signals are observed in the blue region. The negative band is shifted to 682 nm at 293 K. A similar band, albeit at somewhat longer wavelength was observed in a purified PS II preparation by Hinz [28].

Discussion

The method presented here gives a relatively simple procedure for the isolation of an oxygen-evolving reaction-center complex (core complex) from PS II membranes. The preparation of several oxygen-evolving PS II reaction-center complexes retaining manganese and the extrinsic 33 kDa protein has recently been reported [6,7]. The size of our preparation as indicated by the Chl/RC ratio of 45 is less than those that can be derived from the data provided by Ghanotakis et al. [7] and Ikeuchi et al. [6] which correspond to Chl/RC ratios of about 75 and 60, respectively.

The core complex prepared by our procedure is practically free from LHCP and seems to be intact as judged from its photochemistry, oxygen evolution and the polypeptide composition. The structural integrity is also confirmed by the spectroscopic data. The bands that are observed in the low-temperature absorption spectrum of the core and the BBY complex are located at the same position as in the excitation spectrum for PS II fluorescence of intact chloroplasts [17]. In addition, there is a weak band at 693 nm (see below), but the long-wave absorption bands attributed to PS I [17] are lacking, confirming that very little PS I is present in these preparations. Furthermore, the emission and fluorescence excitation spectra indicate a high efficiency of energy transfer between the chlorophylls in the complex, supporting the notion that the isolation procedure did not significantly change the structure of the complex.

The core complex shows a strong enrichment of the band located at 683 nm, which appears to be closely associated with the reaction center of PS II. Its amplitude indicates that at least several transitions contribute to this band, and from the Chl/RC ratio of 45 it follows that 8–10 pigments absorbing near 683 nm are present per reaction center in the core complex if all Q_y transitions have the same dipole strength. The low-temperature absorption spectrum does not resolve any of these components, but the dichroic ratio of the oriented BBY complex suggests that the band is composed of more than one spectrally distinct component, as was also observed by Tapie et al. for a PS II complex from *C. reinhardtii* [24]. One of the components must be P-680, which absorbs at 682.5 nm at low temperature [19], another one is presumably reaction center Pheo *a* [20,21]. The band at 540 nm, which has been attributed to Pheo *a* [8] shows two distinct transitions, indicating that the two Pheo *a* molecules that appear to be contained in the reaction center [29] are spectrally different in the Q_x region, like in purple bacteria.

The emission spectra of the BBY and the core complexes again demonstrate the virtual absence of PS I in these preparations. In contrast to some other PS II preparations [8,22,30,31], the BBY as well as the core complex exhibit clear emission bands near 685 and 695 nm at cryogenic temperatures. These bands, F_{685} and F_{695}, are also observed in the emission spectra of intact chloroplasts and have been attributed to the core of PS II [17]. The weak emission beyond 700 nm probably originates from PS II also [22].

F_{680}, which fluorescence is emitted by LHCP [17,22], is lacking in the core complex, as was to be expected. It is fairly weak in the BBY complex, and can only be discerned as a small shoulder in the emission spectrum at 4 K. This indicates that energy transfer from LHCP to the core complex may be even more efficient in the BBY complex than in intact chloroplasts. In this connection it should be noted that the Chl *b* bands in the LD spectrum, relative to those of Chl *a*, are weaker in the BBY complex than in the corresponding Photosystem II spectrum of intact chloroplasts [12], suggesting a change in the relative orientations of LHCP and the core complex, perhaps by solubilization of part of the lipids [32]. Removal of the lipids may also result in a reduced distance between LHCP and the core complex.

The temperature dependences of F_{685} and F_{695} in our preparations are different from those in intact chloroplasts at temperatures below about 70 K. In chloroplasts the rise in intensity of F_{685} that starts at about 100 K is followed by an increase in the intensity of F_{695} at still lower temperatures, resulting in an about equal intensity of both emission bands at 4 K [22]. In our preparations, however, there was a steady rise of F_{695} with decreasing temperatures, while the intensity of F_{685} stayed approximately constant down to at least 40 K, resulting in an apparent single emission band at 695 nm at 4 K. The reason for this difference is not clear; one might assume that the decrease of the efficiency of energy transfer between the pigments that give rise to F_{685} and F_{695}, respectively, at low temperature, that was postulated to occur in chloroplasts [33] does not occur in our preparations, or, alternatively, that F_{685} is emitted by a different set of pigments at around 100 K and at 4 K, those emitting at 4 K functioning as fluorescent traps in chloroplasts.

The origin of F_{695} has been often debated, and it has variously been assigned to a minor Chl *a* component [22] and to the reaction center of PS II [34]. Our experiments show that its transition dipole makes an angle of less than 55° with the normal to the membrane, in agreement with observations on intact chloroplasts [12] (where a much higher degree of orientation of the membranes could be obtained) and with recent experiments with *C. reinhardtii* [24]. This shows that it cannot be attributed to the Chl *a* transition at 683 nm, which is almost parallel to the membrane, and tends to support the hypothesis [34] that it may represent fluorescence from the photoactive Pheo *a* that functions as an electron acceptor in the reaction center of PS II. The Q_y transition dipole of this Pheo *a* makes a large angle with the membrane [20], and is located at 680–685 nm at room temperature [20,21], so that it may well be hidden behind the 683 nm absorption band. From the deconvolution of the absorption spectrum of the core complex it can be estimated that Chl a_{676} comprises 34% of the total Chl *a* content, corresponding to about 15 Chl a_{676} per RC, if it is again assumed that all Q_y transitions have the same dipole strength. The analysis of the temperature dependences of F_{685} and F_{695} given in Fig. 4 then suggests that the species emitting at 695 nm may occur at a concentration of only one molecule per RC, which would support the conclusion mentioned above.

Tapie et al. [24] recently proposed that F_{695} would originate from a long-wave pigment which can be observed in the LD spectra (Fig. 8). It was further assumed that this pigment would be photoactive Pheo *a* [24]. The amplitude of the 693 nm band (Fig. 2) is in agreement with the assumption that it may represent a reaction-center transition, perhaps of a second Pheo *a* (the orientation of which is not known). However, our results do not indicate that the 693-nm transition would be responsible for the 695-nm emission, First of all, one would then have to assume that the absorption band of the photoactive Pheo *a* shifts by about 10 nm to longer wavelengths upon cooling, and that the Stokes shift is unusually small. Moreover, the temperature equilibrium of F_{685} and F_{695} indicates an energy difference corresponding to 7 nm for the two absorption dipoles. The LD and fluorescence measurements indicate that F_{685} must be emitted by the Chl a_{676}, and this agrees well with a wavelength of 683 nm for the absorption band associated with F_{695}.

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