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Spectroscopic characterization of CP26, a chlorophyll *a* / *b* binding protein of the higher plant Photosystem II complex

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Abstract

A spectroscopic study is presented of the minor chlorophyll *a* / *b* binding protein CP26 isolated from spinach by means of a dodecylmaltoside/betaine washing procedure. The preparations are characterized by a chlorophyll *a*/chlorophyll *b* ratio of 3.3 ± 0.1, and most likely contain 2 chlorophyll *b* (Chl *b*) and 6–7 chlorophyll *a* (Chl *a*) molecules per monomeric protein. Some of the spectroscopic properties of CP26 show strong similarities to those of the major chlorophyll *a* / *b* light-harvesting protein LHC-II, which is in line with the sequence homologies between the two proteins. Spectroscopic differences in the Chl *b* absorption region are caused by the variation in Chl *b* content in both proteins. A strongly blue-shifted Chl *b* band at 637 nm in CP26 has similar linear and circular dichroism properties as the spectral component at 640 nm of LHC-II. It is suggested that these spectral features arise from a conserved Chl *b* molecule, and that the blue shifts are caused by charged amino acids in the vicinity of these Chl *b* molecules. The other Chl *b* band in CP26 is observed at 650 nm. Differences in the Chl *a* absorption region mainly concern the reduced absorption at 670 nm for CP26, whereas a strong band near 675 nm is very similar to the band at 676 nm for LHC II. Tentative assignments of several absorption bands of CP26 and LHC II to specific pigments are made on the basis of the recently reported three-dimensional structure of LHC II and of the primary amino acid sequences of both proteins. In the carotenoid region LHC II and CP26 show slightly different linear and circular dichroism signals. However, the differences are not large enough to exclude a similar arrangement of the two lutein molecules in LHC II and CP26.

Keywords: Photosystem II; Excitation energy transfer; Light harvesting complex; Polarized spectroscopy

1. Introduction

The collective photosynthetic antenna of Photosystem II (PS II) in green plants contains various pigment-protein complexes. Two of these (CP43 and CP47) are closely connected to the reaction center (RC) and do not contain chlorophyll *b* (Chl *b*). In addition, the antenna of PS II is comprised of the chlorophyll *a* / *b* complexes LHC II, CP29, CP26 and CP24 [1]. Light harvesting complex (LHC) II is the most abundant one of these, binding more than half of the total number of chlorophylls in PS II. The proteins CP29, CP26 and CP24 are present in smaller amounts and are therefore designated as 'minor Chl *a* / *b* binding proteins'. The main function of the total antenna is to absorb sunlight and to effectively transfer the excitation energy towards the RC, where it is transformed into useful chemical energy. The major fraction of the radiant energy is absorbed by LHC II. However, the fact that the minor Chl *a* / *b* binding proteins have been conserved in all plants and green algae that have been examined so far indicates that they are also important. One of the most intriguing minor chlorophyll *a* / *b* proteins is CP26, since this protein (formerly known as the '28 kDa' chlorophyll binding protein) co-purifies with PS II [2,3]. A number of experiments by Bowlby et al. [4,5] have suggested that this protein is functionally associated with the acceptor side of PS II,

Abbreviations: *A*, absorption; LD (ΔA), linear dichroism; CD, circular dichroism; LHC, light harvesting complex; PAA, polyacrylamide; DM, dodecylmaltoside.

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and that removal of CP26 disrupts the herbicide sensitive electron transfer from Q_A^- to Q_B and the magnetic coupling between Q_A^- and the non-heme iron.

The determination of the crystal structure of trimeric LHC II at 6 Å resolution by a combination of electron diffraction and electron microscopy [6] revealed a dense packing of the pigments (the chlorophyll mass content was estimated to be 30%), which is favorable for effective light absorption as well as excitation energy transfer. The 6 Å structural model has stimulated a revival of spectroscopic studies on LHC II [7–15]. The recent unraveling of most of the LHC II structure to a resolution of 3.4 Å [16] will stimulate attempts to relate the structure of LHC II to its spectroscopic properties and light-harvesting function.

A monomeric LHC II subunit contains three membrane-spanning helices. At the heart of the complex, two lutein molecules form a cross-brace which seems to be essential for the tertiary structure of the protein. The luteins are close to perpendicular to each other and to the axis that connects the center of both molecules. Their structural role was demonstrated in reconstitution experiments to rebind chlorophyll and carotenoids to the pigment-less polypeptide which was obtained by over-expression in *Escherichia coli* [17,18]. The functional role of carotenoids as quenchers of triplet states which reside mainly on chlorophyll (Chl) *a* and not on Chl *b* has led to the tentative proposal that the 7 chlorophylls that are closest to the lutein molecules are Chl *a*. The remaining 5 Chl molecules that were resolved in the crystal structure were assigned to Chl *b*. Two chlorophyll molecules that so far were not resolved in the crystal structure would bring the total number of chlorophyll in LHC II to 8 Chl *a* and 6 Chl *b* molecules [16].

Spectroscopic measurements on LHC II at cryogenic temperatures revealed 9 spectral forms in the Q_y-absorption region (between 640 and 690 nm) [7,9] and extensive excitonic coupling was observed, in agreement with the dense packing of the pigments. The main absorption band around 676 nm, with an intensity that is approximately equal to that of 4 Chl *a* molecules per monomeric subunit [7], was shown to be polarized essentially parallel to the plane of the trimer [13], whereas the other absorption bands are oriented at larger angles to this plane. By comparison of the spectroscopic properties of monomeric and trimeric LHC II the presence of a 10th band could be resolved for trimeric LHC II [12], whereas recent hole-burning studies revealed an additional band with small amplitude near 680 nm [14].

The structure of LHC II is also of relevance for the minor Chl *a*/*b* binding proteins which show pronounced sequence homology with LHC II (for a review see Ref. [1]), especially in the membrane-spanning helix regions. Several studies indicated the presence of two lutein molecules per minor Chl *a*/*b* binding protein, whereas a lower content of other carotenoids (neoxanthin and violaxanthin) was observed [19,20]. The amino acids that provide the binding sites for seven chlorophylls in LHC II [16] were also found to be present in CP26 [21] with similar amino acids in the immediate environment. In light of the sequence homology and the conservation of a significant number of chlorophyll binding sites [1,16], similarities between the spectroscopic properties of these minor Chl *a*/*b* binding proteins and LHC II might be expected. A comparison of these properties is therefore important for obtaining a better understanding of the structure–spectroscopy relationships in both the major and the minor Chl *a*/*b* complexes.

We have examined the spectroscopic properties of CP26 from spinach, which is virtually identical to CP29-I from tomato [21] and CP26 from maize [22]. The spectroscopic properties of this protein have not been examined in detail before. Absorption and fluorescence spectra at room temperature were presented in Refs. [23,24]. The room temperature absorption spectrum resembles that of LHC II in the Chl *a* Q_y-region (660–690 nm), but shows less absorption in the Chl *b* Q_y-region. Here we describe the results of absorption (*A*), linear dichroism (LD), circular dichroism (CD) and (polarized) fluorescence measurements at 4 K and 77 K, and at room temperature. The spectra are compared to those of LHC II [7,9,12] and implications for the structure–spectroscopy relationship are discussed.

2. Materials and methods

2.1. Purification of CP26

Oxygen-evolving PS II membranes were prepared according to Ref. [25] with modifications described in Ref. [26]. This preparation was then used for the preparation of O_2-evolving reaction center complexes [2], which are devoid of LHC II and other chlorophyll *a*/*b* binding proteins, but which retain CP26 [27,28]. The identity of this species has been confirmed by microsequencing (data not shown). For isolation of CP26 from reaction center complexes, the preparation was solubilized by incubation on ice in the dark at 0.5 mg Chl/ml for 5 min with 0.5% (w/v) dodecylmaltoside (DM) in 20 mM MES-NaOH (pH 6.0), 2 mM $CaCl_2$, 0.4 M sucrose (Buffer A). Insoluble material was removed by centrifugation (40 000 × *g*, 10 min). The 22 kDa and 10 kDa proteins were removed by cation exchange chromatography using S-Sepharose Fast-Flow (HR-10/10 column) and a Pharmacia FPLC system. The column was equilibrated with several column volumes of Buffer A containing 0.05% DM before

the reaction center complex solution was applied at a flow rate of 1 ml/min. Material eluting from the column was applied directly to an anion exchange column (Q-Sepharose Fast-Flow, HR-10/10 column) attached in series and equilibrated with buffer A. Trace amounts of free pigments were washed from the column with several column volumes of buffer A containing 0.05% DM. When the absorbance had returned to baseline, the flow-rate was reduced to 0.5 ml/min and the column was washed with 10–15 ml of a solution of Buffer A supplemented with 4% (v/v) betaine, 0.5% (w/v) DM, and 10% (v/v) Buffer B (0.1 M MES-NaOH (pH 6.0), 0.2 M $CaCl_2$, 0.05% DM), which eluted CP26. Solid sucrose was added to the Chl-containing eluate fractions to a final concentration of 0.4 M and samples were stored at −60°C until used. This method produces protein which is judged to be > 95% pure based on gel electrophoresis [29].

2.2. Spectroscopy

For the characterization of CP26 at low temperature by absorption, fluorescence and circular dichroism spectroscopy, the complex was solubilized in 84% (v/v) glycerol (Merck) and then frozen to 77 K using an Oxford cryoholder (Oxford Instruments, UK) or to 4 K using a He-flow cryostat (Oxford). Absorption and fluorescence spectra were recorded in 1 × 1 cm acryl cuvettes; for CD measurements samples were frozen in an open quartz 1 × 1 × 0.5 cuvette as described in Ref. [7]. All measurements were performed as described previously [7]. For recording LD spectra the complex was oriented in polyacrylamide (PAA) gels containing 55% (v/v) glycerol, 14.5% (w/v) acrylamide/0.5% *N,N'*-methylbisacrylamide) which were compressed in two perpendicular directions (*x*- and *y*-axis) with a factor of 1.25 and the gels expanded along the *z*-axis with factor of $(1.25)^2$. The gels were polymerized with 0.05% (w/v) ammonium persulfate and 0.03% Temed (Sigma).

3. Results

3.1. Chl a / Chl b ratio

For CP26, significantly different values have been reported for the number of chlorophylls: 7 Chl *a* and 4 Chl *b* [20], 9 Chl *a* and 5 Chl *b* [19], 6 Chl *a* and 3 Chl *b* [22], whereas Chl *a*/Chl *b* ratios of 2.9 [30] and 4–5:1 [31] have also been reported. We determined the Chl *a*/Chl *b* ratio according to the method of Porra et al. [32] after dissolving CP26 in an 80% acetone mixture. The ratio was found to be 3.3 ± 0.1. Assuming an integral number of Chl *b* molecules per CP26 complex this ratio implies either 2 or 3 Chl *b* pigments in view of the total number of chlorophylls associated with CP26 [19,20,22]. A value of 3 would lead to a number of 10 Chl *a* molecules which is higher than any number determined so far [19,20,22]. Therefore, the presence of 2 Chl *b* molecules and 6 or 7 Chl *a* molecules seems most probable.

3.2. Absorption

A comparison of the absorption spectra of CP26 at room temperature (—) and 77 K (---) is presented in Fig. 1a; the Q_y-region is expanded in Fig. 1b. The 4 K

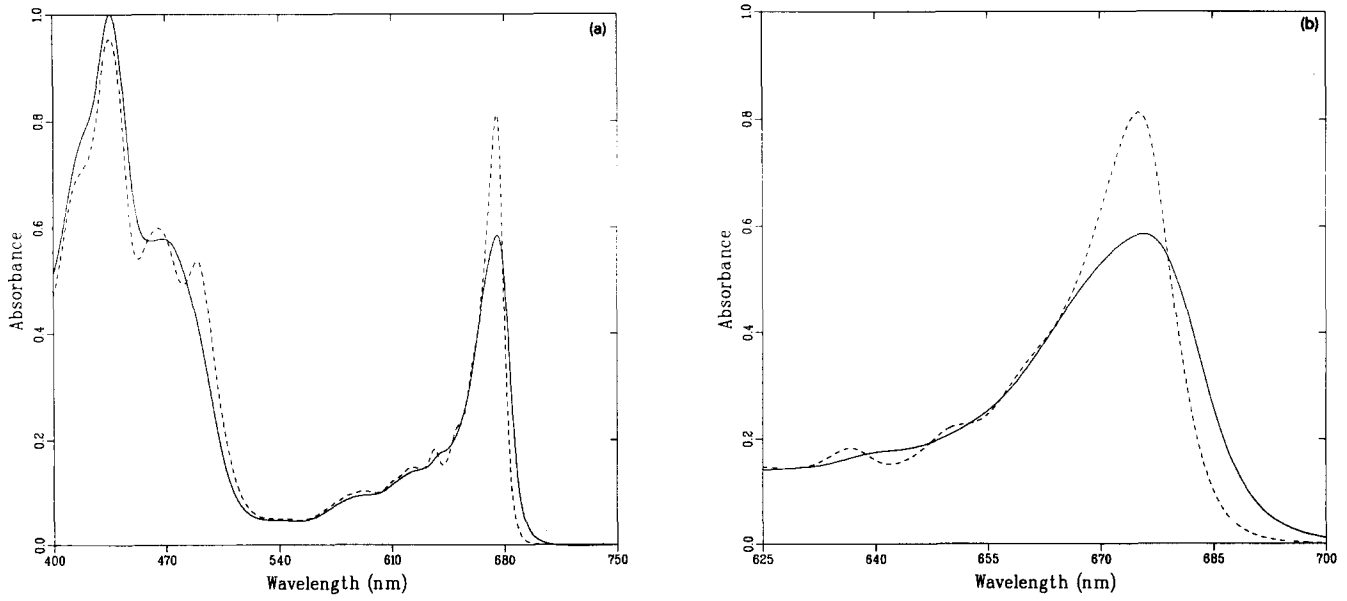

Fig. 1. (a, b) Absorption spectra of CP26 in a glycerol-buffer mixture recorded at room temperature (—) and 77 K (---) with an optical bandwidth of 0.5 nm.

spectrum is not significantly different from the 77 K spectrum (not shown). A strong band associated with most of the Chl *a* dipole strength is observed near 676 nm at room temperature. At 77 K this main band has sharpened asymmetrically towards the blue; this is the origin of the significant increase of the absorption in the peak, which is now located at 675 nm. A similar phenomenon was observed for LHC II [33], and the main band of CP26 is very similar to that at 676 nm in monomeric and trimeric LHC II [7,12]. A striking difference from the LHC II spectrum is the absence of a distinct 670 nm band. LHC II further shows a clear band at 661 nm (77 K) whereas only a shoulder near this wavelength is observed for CP26. The intensity of the Chl *b* band at 650 nm is much smaller for CP26 because of the reduced number of Chl *b* molecules. The Chl *b* peak near 637 nm (77 K) is reminiscent of the 640 nm band for LHC II [7,12]. In the Soret region Chl *a* produces a band at 437 nm (as for LHC II), whereas the band at 464 nm (77 K) is due to Chl *b* and carotenoids (mainly lutein). The peak at 491 nm is assigned to carotenoids. Note that between 450 nm and 500 nm the absorption for LHC II is much stronger, which is at least partly due to the larger number of chlorophyll *b* molecules [7]. For LHC II, peaks are observed at 459, 473 and 484 nm, indicating that the carotenoid absorption characteristics are also different. Although both proteins bind two lutein molecules per monomer, the amount of additional carotenoids might differ.

3.3. Linear dichroism

The LD spectrum of CP26 at 77 K is given in Fig. 2a and an expansion of the Q_y-region is presented in Fig. 2b. The shape of the LD spectrum resembles that of monomeric and trimeric LHC II with a strongly positive peak near 676 nm [7,12]. The LD near 650 nm is different from that of monomeric LHC II, which could be anticipated due to the lower Chl *b* content of CP26. However, the positive Chl *b* band at 638 nm is similar to the LD band at 640 nm for LHC II. The fact that the 638 nm LD band is slightly red-shifted with respect to the 637 nm absorption band may be due to some spectral overlap with the Q_x-band of Chl *a* [34,35] which shows negative LD. The LD of the chlorophylls is low in the Soret region of the spectrum. The positive and negative bands at 499 and 478 nm are presumably due to carotenoids. The opposite signs in the carotenoid LD spectrum indicate the presence of more than one carotenoid molecule. Carotenoids with slightly different absorption spectra and different orientations with respect to the orientation axis can explain this observation. However, it is also possible that two closely spaced carotenoids show excitonic interactions leading to two perpendicular carotenoid bands. For instance, an arrangement of two lutein molecules in the heart of CP26 like in LHC II could cause such an interaction. Their nearly perpendicular arrangement (see above) would lead to two exciton bands which are separated by only a small energy gap and therefore these exciton bands would largely overlap. This would explain why the LD signals are not very pronounced. The LD of LHC II in the carotenoid region is different as was shown in Ref. [7] but the average carotenoid LD in both proteins is small. Therefore, the observed differences of the LD signals in both systems may be caused by small differences in the arrangement of lutein molecules in both proteins. Note that other carotenoids

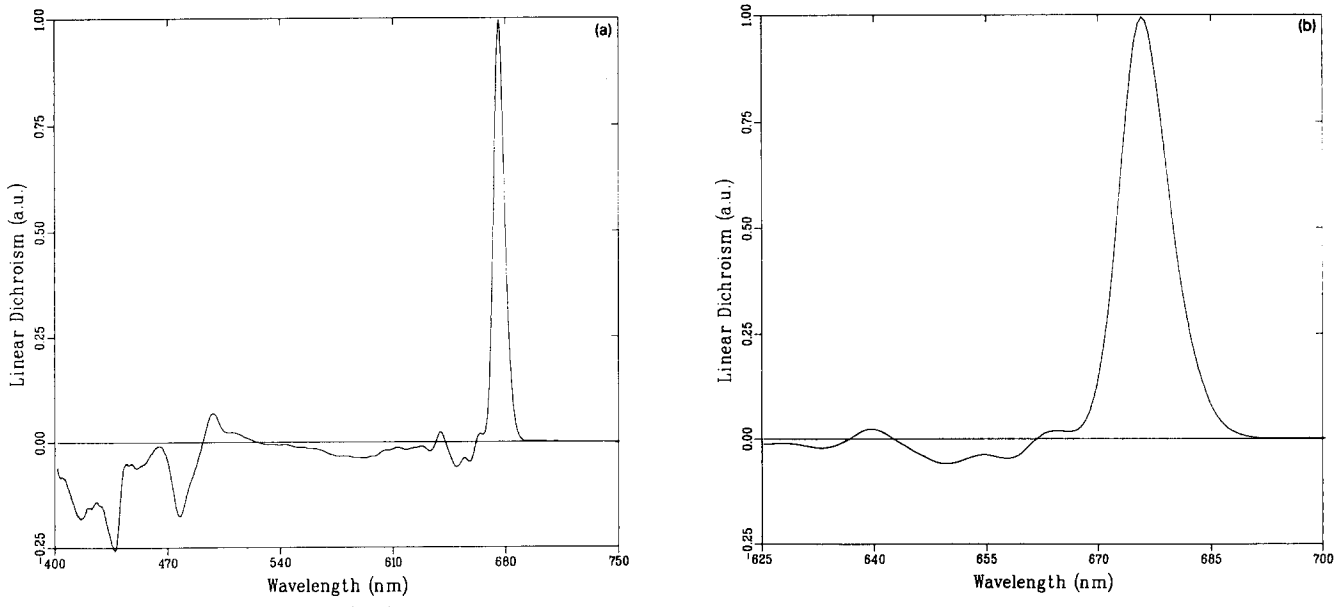

Fig. 2. (a, b) LD spectra of CP26 recorded at 77 K with an optical bandwidth of 3 nm.

also can contribute, thereby complicating the interpretation.

3.4. Circular dichroism

The CD spectrum of CP26 at room temperature is given in Fig. 3a. The CD in the Q_y-region is shown in Fig. 3b (recorded at 4 K). The global characteristics of the CD spectrum in the Chl *a* Q_y-region are again similar for CP26 and monomeric and trimeric LHC II [7,12], i.e., positive on the short wavelength side and strongly negative at the red side near the main absorption band. The intensity of the negative peak at 678.5 nm is of a similar magnitude to that of monomeric and trimeric LHC II [12]. Note that the position of the negative peak is slightly blue-shifted with respect to the room temperature spectrum. It might be related to the asymmetric sharpening of the absorption spectrum in this wavelength region that occurs upon decreasing the temperature. Note that in the Chl *b* region the spectra differ at room temperature and 77 K. This might be partly due to some uncertainties in the baseline correction. The fact that the strongly negative peak near 650 nm for LHC II is not observed for CP26 is probably related to the reduced number of Chl *b* molecules in CP26. However, the negative Chl *b* band near 637 nm is again similar to the negative 640 nm band for LHC II. The fine structure in the carotenoid region above 470 nm (negative peaks at 501 and 473 nm) points to excitonic interactions between the carotenoid molecules and/or Chl *b* molecules. Circular dichroism experiments on different aggregation states of LHC II were described in Ref. [36] and a similar CD spectrum was only observed in the same wavelength region for LHC II in high detergent concentrations, under which conditions LHC II is probably monomeric [12].

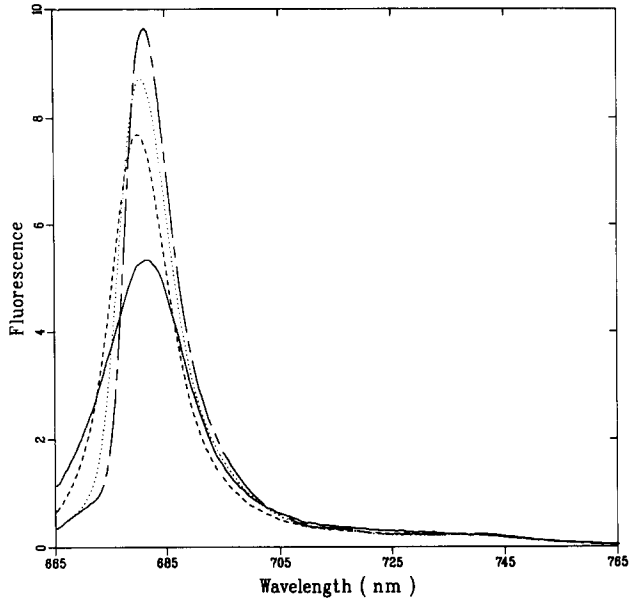

Fig. 4. Fluorescence spectra of CP26 at different temperatures (293 K (—), 150 K(---), 50 K (···) and 4 K (– - –). The sample was excited at 436 nm and an optical bandwidth of 18 nm was used. The spectra were recorded using an optical bandwidth of 4 nm.

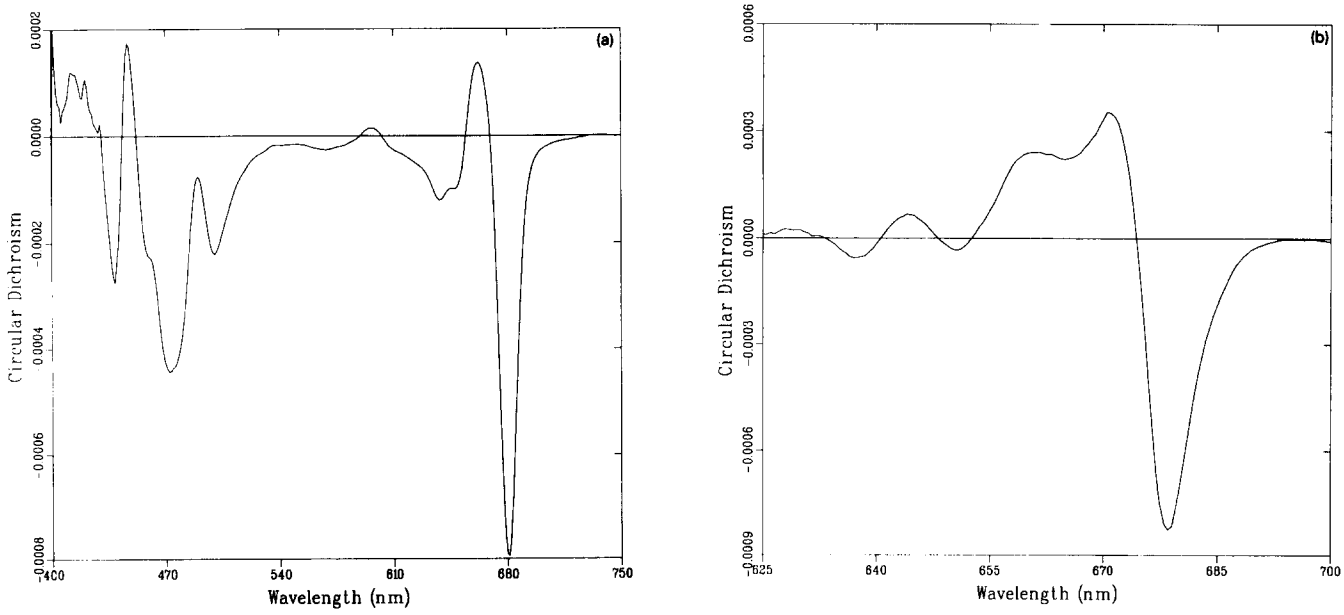

Fig. 3. (a) CD spectrum of CP26 at 293 K recorded with an optical bandwidth of 6 nm. The spectrum has been smoothed to reduce the noise below 450 nm. The optical density at 676 nm is 0.8. (b) CD spectrum of CP26 in a glycerol-buffer mixture at 4 K recorded with an optical bandwidth of 6 nm. The optical density at 676 nm is 0.6.

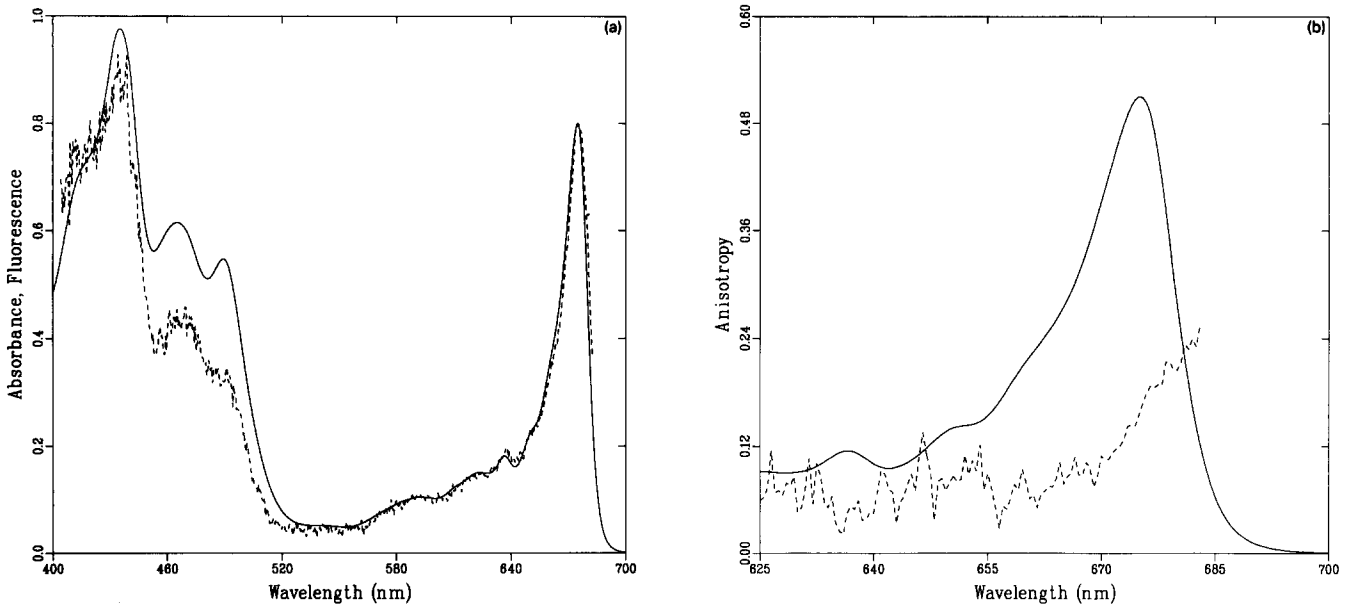

Fig. 5. (a) Excitation (---) spectrum of CP26 at 4 K measured at 692 nm with an optical bandwidth of 12 nm. Excitation was performed using an optical bandwidth of 4 nm. The absorption spectrum of CP26 at 4 K (—) was recorded with an optical bandwidth of 4 nm. (b) Fluorescence anisotropy of CP26 recorded under identical conditions as given in (a) (---). The absorption spectrum of CP26 at 4 K (—) was recorded with an optical bandwidth of 4 nm.

3.5. Fluorescence

The fluorescence spectra of CP26 at different temperatures (293 K (—), 150 K(---), 50 K (· · ·) and 4 K (– - –) are presented in Fig. 4. The sample was excited at 436 nm (Chl *a* band). The fluorescence spectrum narrows at lower temperatures. At 4 K a weak shoulder, probably due to free Chl *a* is observed near 670 nm. The maximum of the fluorescence blue-shifts from 682.5 to 680.5 nm upon cooling from 293 K to 100 K after which it red-shifts again to 682 nm at 4 K. The initial blue-shift corresponds to the asymmetric sharpening of the main absorption band towards the blue. The estimated full width at half maximum (fwhm) decreases continuously from approximately 20 nm at 293 K to 9 nm at 4 K. The fwhm at 4 K is larger than that of trimeric LHC II (5.5 nm) [9] but similar to that of monomeric LHC II (10 nm) [8].

The 4 K excitation spectrum of the fluorescence at 692 nm is shown in Fig. 5a (dashed line). Since the absorption in the peak was low (0.05), the excitation spectrum can directly be compared with the absorption spectrum which is also given in Fig. 5a (full line). Above 540 nm (the chlorophyll absorption region) both spectra are essentially identical, indicating > 90% efficient energy transfer from all chlorophyll molecules to the emitting species. In the carotenoid region, however, there is a clear difference, indicating that only some of the carotenoids transfer their excitation energy efficiently to the chlorophyll molecules. Almost perfect energy transfer would be expected if lutein is arranged similarly in LHC II and CP26. The most likely explanation of this result is that other carotenoid molecules are present that transfer their excitation energy not very effectively to the chlorophyll pigments. In Refs. [19,30] it was reported that besides lutein also neoxanthin and violaxanthin are present in CP26. If these carotenoids are not in close contact with chlorophyll molecules then a reduced transfer efficiency is expected.

The excitation anisotropy spectrum in the Q_y-region is given in Fig. 5b. Again the spectrum shows similarities to that of LHC II [7], i.e., the anisotropy is close to zero below 660 nm and increases upon going to the red. The absolute value of the anisotropy in the red part of the spectrum is higher for CP26 than for LHC II but still lower than the theoretical maximum value of 0.40, demonstrating that even in the red wing of the absorption spectrum depolarization due to energy transfer takes place.

4. Discussion

4.1. Chlorophyll b

In the low-temperature absorption spectra of CP26, three bands are observed that could originate from Chl *b*: 659.5, 650 and 637 nm. The latter two cannot be assigned to Chl *a* since they absorb too far to the blue and we therefore assign them to Chl *b*. The 659.5 nm band is due either to Chl *b* or Chl *a*, but since it is

more likely that only two Chl *b* molecules are present (see above) we suggest that this band arises from Chl *a*.

In LHC II most of the Chl *b* absorption is centered around 649 nm, but a band is also observed at 640 nm for trimeric LHC II [7,9,12,13,15] and for monomeric LHC II obtained by exposure to elevated concentrations of octylglucoside [12]. Like the 637 nm band in the present study, the LHC II 640 nm band generates small positive LD and small negative CD. These similarities between the 637 nm band of CP26 and the 640 nm band of LHC II (strong blue-shift, small positive LD, small negative CD) strongly suggest that both bands arise from a Chl *b* molecule whose orientation and ligation is similar in both proteins. The absorption maximum of this band is strongly blue-shifted with respect to that of free Chl *b* in apolar solutions [37,38]. Since in the crystal structure of LHC II no pigments are in sufficiently close contact to induce such a strong blue-shift through excitonic coupling, we ascribe the origin of the 637 nm band to specific interactions with charged amino acid groups in the vicinity of the corresponding pigment [39].

In trimeric and monomeric LHC II at least three Chl *b* molecules per monomer contribute to the band at 649 nm [7,9,12], and a comparison with the 650 nm band of CP26 is less straightforward than for the 640 nm species. Two of the proposed binding sites for Chl *b* (Chl b_3 and b_5) in LHC II [16] are also present in CP26 [21], and it is tempting to speculate that these sites also bind Chl *b* in CP26. Because of the strong sequence homology and the predicted structural homology around the binding sites [40] it is reasonable to assume similar spectroscopic properties of the corresponding Chl *b* pigments, and in that case Chl b_3 or b_5 would be responsible for the 640 nm band in LHC II. For Chl b_5 an arginine-glutamate pair is juxtaposed on the chlorophyll, whereas for Chl b_3 no charged groups are close [16], suggesting that Chl b_5 is the most likely candidate for the 637–640 nm absorption band in LHC II and in CP26. This would imply that Chl b_3 is responsible for the 650 nm absorption band in CP26.

4.2. Chlorophyll a

The majority of the absorption of CP26 in the Chl *a* region resides in the main band at 675 nm (77 K). As in the case of LHC II, this band has a composite character, since the reduced LD exhibits a peak at longer wavelength (676 nm) that decreases steeply upon going to 670 nm, indicating the presence of a band near 670 nm with less LD. Although such a band cannot readily be observed in the absorption spectrum of CP26 it gives rise to a positive CD band (see Fig. 3b). The presence of a band near 670 nm was already proposed in Ref. [23]. The LD of the main band is strongly positive whereas the CD is strongly negative. Together with the red shift of this absorption band with respect to that of free Chl *a*, these dichroism features are strikingly similar to those of monomeric and trimeric LHC II [7,12], which points to a similar origin in all cases. As with LHC II, part of the red absorption band shifts to shorter wavelengths upon cooling [33]. From a Gaussian decomposition of the absorption spectrum of CP26 at room temperature, a relatively weak band at 683.5 nm was concluded to be present [23]. The absorption intensity around this wavelength decreases substantially upon cooling whereas the absorption around 675 nm increases. These changes would be in line with a blue shift of the red-most absorption band. Other related features observed for both proteins include the positive shoulder near 664 nm in the LD spectrum, the negative LD just below 660 nm (which is probably due to Chl *a* in CP26) and the positive CD above 660 nm. The position of the Chl *a* fluorescence band is also similar for CP26 and LHC II [7,12]. Upon cooling to 100 K, the fluorescence maximum shifts to the blue, which correlates with the blue shift of the red-most absorption bands, from which the main part of the fluorescence arises. Below 100 K the fluorescence begins to red-shift, demonstrating that below this temperature thermal equilibration prevails.

In addition to these similarities there are some differences between the spectroscopic properties of both proteins. Whereas LHC II shows a distinct absorption peak near 670 nm, this peak is strongly reduced in CP26. Therefore, it is likely that Chl *a* binding sites that are present in LHC II are absent in CP26 and that (some of) the chlorophylls bound to these sites in LHC II give rise to the 670 nm band. In view of the similar LD spectra of LHC II and CP26, these pigments must generate negligible LD. On the other hand, it is possible that LHC II contains several extra chlorophyll molecules that are not resolved in the crystal structure (for instance due to mobility or disorder) and these could, in principle, be responsible for the 670 nm band. However, the fact that trimeric LHC II shows coupling between the 667 and 673 nm bands (contributing to the overall 670 nm absorption band) in the CD-spectrum [8] suggests that the corresponding pigments have well-defined orientations and positions in the trimer. Therefore, the 670 nm band most likely corresponds to pigments that are also observed in the crystal structure.

Five of the presumed binding sites for Chl *a* (a_1, a_2, a_3, a_4 and a_5) in LHC II are also present in CP26 and similar amino acid residues are present in the vicinity of these binding sites. It is therefore not unreasonable to ascribe the common Chl *a* absorption band of CP26 and LHC II near 675–676 nm and the similar LD and CD features for a large part to these five pigments.

This would imply that pigments a_6 and a_7 cause a large part of the 670 nm absorption.

4.3. Carotenoids

The dominating carotenoid species in CP26 is lutein and two of these molecules are present per complex [19,20,30]. For LHC II the essential structural role of lutein molecules has been demonstrated [17,18] and the crystal structure reveals that two lutein molecules are present in the heart of the LHC II structure [16]. Their important structural and functional (as quenchers of Chl *a* triplets) role was discussed elaborately [16]. In CP26 these luteins could have a similar role and arrangement. The low average LD and the excitonic interactions (as revealed by the CD) of the carotenoids could be consistent with this proposal. However, the presence of only two luteins in a similar arrangement as in LHC II cannot explain the relatively low average transfer yield of excitation energy from carotenoids to chlorophylls, and additional carotenoids would have to be invoked. This would be in accordance with the pigment analysis performed in Refs. [19,20] where the presence of neoxanthin and violaxanthin was also observed.

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