Fucoxanthin-Chlorophyll Proteins in Diatoms: 18 and 19 kDa Subunits Assemble into Different Oligomeric States[†]

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ABSTRACT: Fucoxanthin−chlorophyll proteins were purified from the centric diatom *Cyclotella meneghiniana*. Two major fractions were observed that differed in their polypeptide composition and oligomeric state. Trimers consist of mainly 18 kDa polypeptides. Higher oligomers are tightly assembled from different trimers, which contain mostly 19 kDa subunits. In both oligomeric states, the excitation energy coupling between fucoxanthin and chlorophyll *a* was preserved, and chlorophyll *c* was shown to transfer energy efficiently to chlorophyll *a*. Circular dichroism spectra showed close interaction between fucoxanthin and chlorophyll *a*, and different chlorophyll *a* molecules were demonstrated to interact excitonically. The assembly of trimers of antenna proteins with a distinct subunit composition into higher oligomeric states was not reported so far and differs from the situation found in higher plants. The differences in the supramolecular structure of the fucoxanthin-chlorophyll proteins reflect the dissimilar arrangement of the thylakoid membranes in diatoms, which lack the grana-stroma distinction.

Diatoms (*Bacillariophyceae*) belong to an eukaryotic group of algae, the heterokontae, which evolved during a secondary endosymbiosis event. Heterokont algae were formed when a heterotrophic host engulfed a eukaryote that already possessed chloroplasts, resulting in a chloroplast surrounded by four membranes. Recent work suggests that the ancestor of their plastids is closely related to rhodophytes (red algae) (*1*−*3*). The diatom photosynthetic apparatus is generally believed to be homologous to other eukaryotic thylakoid systems with the same light-driven reactions and essentially the same electron transport chain. Although the diatoms, and the heterokontae in general, are of considerable ecological importance, they are poorly studied as compared to higher plants, green algae, and photosynthetic bacteria.

Like higher plants, diatoms possess only membrane-intrinsic antenna proteins. These are highly homologous to other light-harvesting complexes (LHC) and also belong to the so-called *cab*-protein family (*4*−*6*). The homologies are significant especially in helix one and three of the three predicted transmembrane α-helices. According to predictions from the sequences or estimates from denaturing gel electrophoresis (*7*−*13*), the polypeptides are considerably smaller in size (in the range of 17−23 kDa), and the shorter N- and C-terminus and smaller loops render the proteins more hydrophobic as compared to other LHCs. The antenna complexes bind chlorophyll (Chl)[1] *a* like all *cab*-proteins, and some of the Chl *a* binding sites are conserved. Otherwise, pigmentation differs considerably. The accessory Chl *b* is replaced by Chl *c*, a chlorophyll lacking the phytolester. Instead of lutein and neoxanthin, fucoxanthin and diadinoxanthin are bound, which are both responsible for the brown color of the algae. Because fucoxanthin is the most prominent carotenoid in these organisms, the antenna proteins are usually called fucoxanthin-chlorophyll proteins (FCP).

Further differences between higher plants or green algae and heterokonts concern the number of functionally different FCPs and their interaction with other proteins of the thylakoid membrane. LHCII in higher plants is a trimeric complex in vivo and is accompanied by several monomeric, minor LHCs in the vicinity of photosystem II. Photosystem I is surrounded by dimers of LHCI. Usually one major FCP has been purified from diatoms (*8, 14*) and the related brown algae (*14*−*19*) and was interpreted as an antenna for both photosystems. Although several genes are coding for FCPs, no antenna specific for PSI was found so far. All antenna complexes copurified with PSI from diatoms or brown algae were identical to the main antenna complex (*14, 20*). Using antibodies directed against the minor LHC in green plants, some of the diatom FCPs were tentatively assigned to minor antenna proteins (*11*), but this has still to be confirmed by more thorough biochemical studies.

Another unsolved question relates to the oligomeric state of the FCP in vivo. LHCII is known to be trimeric, whereas LHCI was shown to be dimeric. The oligomeric state of diatom FCPs is unknown so far. There are reports of heptameric assemblies from a brown alga deduced from Svedberg coefficient determination and electron microscopy (*17, 21*). According to the molecular weight reported, the heptameric assemblies most probably consist of seven trimers. More recently, a fraction, which represents FCP trimers as well, was purified using sucrose gradients from the brown alga *Pelvetia canaliculata* (*19*).

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[1] Abbreviations: CD, circular dichroism; Chl, chlorophyll; DDM, dodecyl maltoside; FCP, fucoxanthin-chlorophyl protein; LHC, light-harvesting complex; PS, photosystem; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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In *Cyclotella cryptica*, a centric diatom, up to 23 gene copies encoding FCPs were detected, and eight different genes were characterized (*12*, *13*, *22*). These genes were derived from cDNA clones, which implies that they are transcribed in vivo. *Fcp1*, *fcp2*, *fcp3*, and *fcp4* are coding for proteins of about 18 kDa, and *fcp5*, *fcp6*, and *fcp7* encode FCPs of about 19 kDa. The gene product of *fcp12* is the largest with approximately 22 kDa. It is still unknown whether these different proteins show variations in pigmentation, oligomeric state, or location in the thylakoid membrane. In this study, intact FCPs were isolated in different oligomeric states from the related species *Cyclotella meneghiniana*, and a preferential organization of the 18 kDa proteins into trimers and of the 19 kDa polypeptides into higher oligomeric states is reported.

MATERIALS AND METHODS

Growth Conditions. The diatom *C. meneghiniana* (Culture Collection Göttingen, strain 1020−1a) was grown in batch cultures in ASP-2 medium (*23*) supplemented with 1 mM silica at 20 °C under a 16 h light (40 μmol m^{-2} s^{-1}) to an 8 h dark cycle.

Preparation of FCP. Thylakoid membranes were isolated according to the method described by Büchel and Wilhelm (*24*) with slight modifications to reduce chlorophyllase activity. Cells were harvested by centrifugation and resuspended in buffer A (10 mM Mes pH 6.5, 2 mM KCl, 5 mM EDTA, 1 M sorbitol), and silica crystals were removed by slow centrifugation. All following steps were carried out in dim light and at 4 °C. Cells were broken using a cell disrupter (Constant Cell systems) at 250 kPa. Cell debris and unbroken cells were removed during a slow spin (1000*g* for 10 min), and membranes were pelleted by 1 h of centrifugation at 75 000*g*. The membrane fraction was then washed using buffer B (10 mM Mes pH 6.5, 2 mM KCl, 5 mM EDTA) by a further centrifugation step (20 min, 40 000*g*), resuspended in a little amount of buffer C (10 mM Mes pH 6.5, 2 mM KCl), and frozen until use.

Thylakoids were solubilized at 0.125 mg Chl *a*/mL with 10 mM β-1,4-dodecyl maltoside (β-DDM, β-DDM/Chl *a* = 41:1, w/w) for 20 min on ice and loaded on top of a continuos sucrose gradient achieved by a freeze−thaw cycle of a solution of 19% (w/v) sucrose in buffer C. Separation was carried out by centrifugation using a swing-out rotor at 200 000*g* for 16 h, which was shown to be sufficient to reach equilibrium.

Bands of brown color were harvested and concentrated using Amicon filtrating devices with a cutoff of 30 kDa. For further purification, they were loaded onto a Superose6 (Pharmacia) gel filtration column attached to an Äkta-FPLC system. Gel filtration was carried out using buffer C supplemented with 0.03% (w/v) β-DDM. Chromatograms were recorded at 280 and 440 nm.

Spectral Characterization. Chlorophyll determination was carried out according to Jeffrey and Humphrey (*25*). For absorption spectra, a Perkin-Elmer spectrophotometer (Lambda Bio 40) was used with 1 nm band-pass and 1 cm optical path length at a Chl *a* concentration of 1.5 μg/mL. Fluorescence excitation spectra at an emission wavelength of 671 nm were recorded with a 3 mm optical path length at a Chl *a* concentration of 1.5 μg/mL in a Hitachi F-4500 fluorometer at room temperature. Excitation and emission bandwidths were 2.5 and 5 nm, respectively. Correction for the excitation side was carried out using a rhodamine B spectrum as reference. Fluorescence emission spectra at room temperature were recorded at an excitation wavelength of 486 nm and excitation and emission bandwidths of 5 and 2.5 nm, respectively. All other parameters were the same as used for the excitation spectra. Spectra of circular dichroism (CD) were measured at 4 °C using 1 cm optical path length, 2 nm bandwidth, and 2 s response time in the range of 370−600 nm and 8 s response time between 600 and 700 nm in a Jasco 810 CD spectrometer. To improve the signal-to-noise ratio, all spectra presented are averages of 10 sequentially recorded spectra.

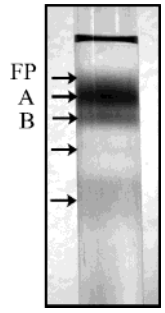

FIGURE 1: Sucrose gradient centrifugation of *C. meneghiniana* thylakoids solubilized with β-DDM. Arrows indicate pigmented bands.

Characterization of the Oligomeric State and Polypeptide Composition. To analyze the oligomeric state of the FCPs, analytical gel filtration was carried out using a Superose6 column (Pharmacia) attached to a SMART system (Pharmacia). Run conditions were identical to the preparative gel filtration described previously. For calibration, monomers and trimers of LHCII of pea (kindly provided by J. Standfuss, MPI of Biophysics, Frankfurt), solubilized in β-DDM and separated by sucrose density centrifugation, were used.

To check whether the oligomeric state is dependent on the presence of phospholipids, 100 μL of trimers at a Chl *a* concentration of 10 μg/mL were incubated for 20 min at 25 °C with 10 units of phospholipase A_2 (Sigma), and their oligomeric state was checked afterward by gel filtration and nondenaturing gel electrophoresis.

Nondenaturing gels based on a Tris-borate buffer system according to Wiedemann et al. (*26*) were used with slight modifications and stained with Coomassie Brilliant Blue G-250 (Serva, 0.25% (w/v) in 10% acetic acid (v/v)). Denaturing gels were run according to Schägger and von Jagow (*27*) and Coomassie stained.

RESULTS AND DISCUSSION

Purification of the FCPs and Determination of their Oligomeric State. A crude thylakoid membrane fraction was solubilized using β-dodecyl maltoside (β-DDM) to purify FCPs from the diatom *C. meneghiniana*. Five colored bands were obtained after sucrose density centrifugation (Figure 1). The lower two bands were green and most probably contained the photosystems. They were not considered further. The three upper bands of brownish color (fractions FP, A, and B) were harvested. Fractions A and B were characterized with respect to their polypeptide composition, spectroscopic characteristics, and oligomeric state.

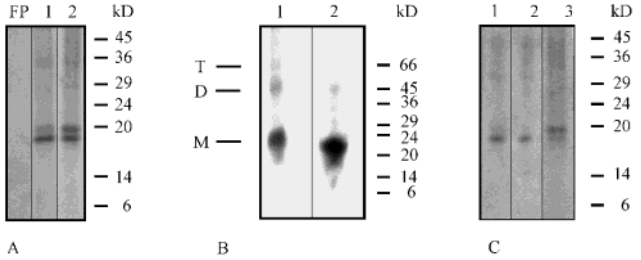

FIGURE 2: Gel electrophoresis of FCPs. Molecular weights are indicated in each panel. (A) SDS−PAGE of fractions harvested from sucrose gradients such as shown in Figure 1. Lane FP represents free pigment, lane 1 shows fraction A, and lane 2 shows fraction B, respectively. (B) Coomassie-stained, nondenaturing gel of trimeric FCP isolated by gel filtration (lane 1) and after treatment with phospholipase (lane 2). Trimers (T), dimers (D), and monomers (M) are indicated. (C) SDS−PAGE of trimers isolated from sucrose gradient fraction A (lane 1), trimers from fraction B (lane 2), and higher oligomers from fraction B (lane 3).

Figure 2A shows a denaturing SDS−PAGE of the three gradient fractions. The olive-brown FP fraction did not contain any protein (Figure 2A, lane FP) and was identified as free pigment released during the isolation procedure. Both fractions A and B contained two polypeptides of 18 and 19 kDa (Figure 2A, lanes 1 and 2), the size known for the apoproteins of FCPs in *C. cryptica* (*12*). Fraction A contained more of the smaller polypeptide, whereas both polypeptides were present in about equal amounts in fraction B.

Figure 3A gives the absorbance spectra of the two gradient fractions. Both are room-temperature spectra typical for FCPs from either diatoms or brown algae (*8*, *14*, *17*, *28*). The Q_y band of Chl *a* in these complexes absorbs at 672 nm, whereas the Q_y absorbance of Chl *c* is only visible as a small peak at 636 nm. As described earlier, there is a strong absorbance between 475 and 565 nm due to the carotenoids bound to the FCPs, mainly fucoxanthin and diadinoxanthin. Whereas both spectra are identical in the red wavelength region, there are slight differences in carotenoid absorption. The most pronounced difference is a shoulder at 486 nm only visible in the spectrum of fraction A. In diatoms, the content of diadinoxanthin varies as an adaptation mechanism to different light intensities (*29*). The shoulder at 486 nm might reflect an increased amount of this carotenoid in fraction A as compared to fraction B. In addition, fraction A might be slightly contaminated by xanthopyll that is not bound to protein and thus shifted in absorption. The fluorescence excitation spectra show that most carotenoids found in either fraction transfer excitation energy to Chl *a* (Figure 3B). The spectra of the two gradient fractions closely resemble each other. However, the shoulder at 486 nm found in the absorbance spectrum of fraction A is not visible in the fluorescence spectrum and is thus due to a slight contamination by decoupled carotenoid. Chl *c* transferred the absorbed energy efficiently to Chl *a* in fraction A as well as in fraction B. This is demonstrated by the fluorescence emission spectra recorded upon excitation at 468 nm (i.e., into the Soret band of Chl *c* (Figure 3C)). No Chl *c* emission at 645 nm is visible, in contrast to the spectrum of free pigments recorded under the same experimental conditions (Figure 3C).

In Figure 4A, the spectra of circular dichroism (CD) of the respective fractions are given. As expected, no pronounced CD signals are visible in the spectrum of free pigments. Fraction A was characterized by a split signal in

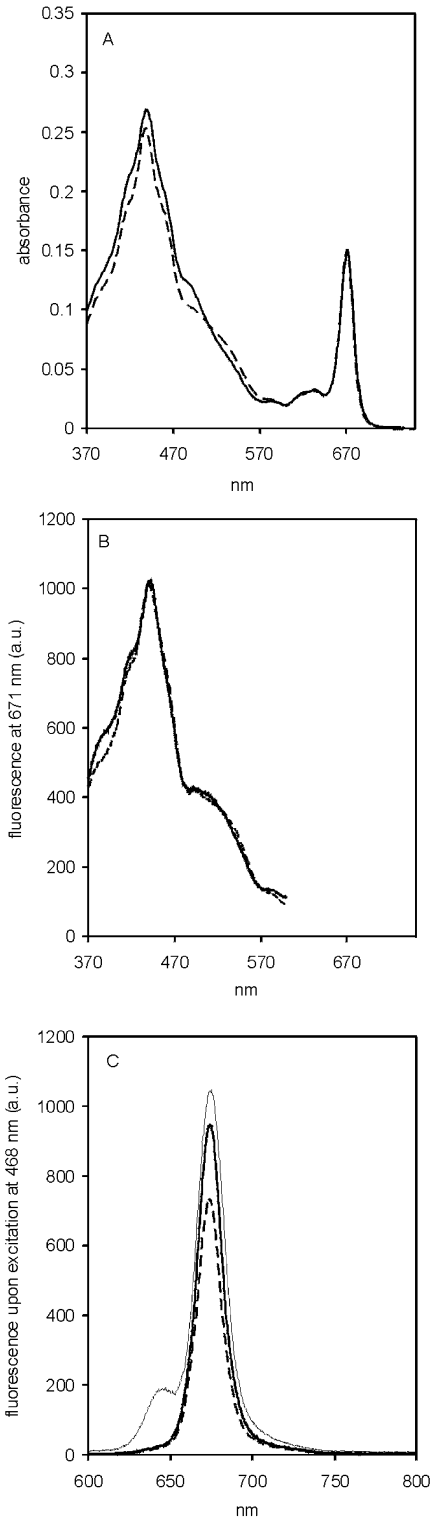

FIGURE 3: Spectra of FCPs of fraction A (solid line) and fraction B (dashed line) from the sucrose gradients. (A) Absorbance spectra. (B) Fluorescence excitation spectra recorded at 671 nm. (C) Fluorescence emission spectra scanned upon excitation at 468 nm. In panel C, the fluorescence emission spectrum of free pigments recorded under the same conditions is shown as well (thin line).

the red wavelength region with negative peaks at 674 and 663 nm separated by a more positive signal at 671 nm. Fraction B had a much smaller negative peak at 677 nm and the most pronounced CD signal in the red at 663 nm. A huge CD signal in the blue wavelength region with a shoulder at 462 nm, a negative peak at 480 nm, and a positive peak at

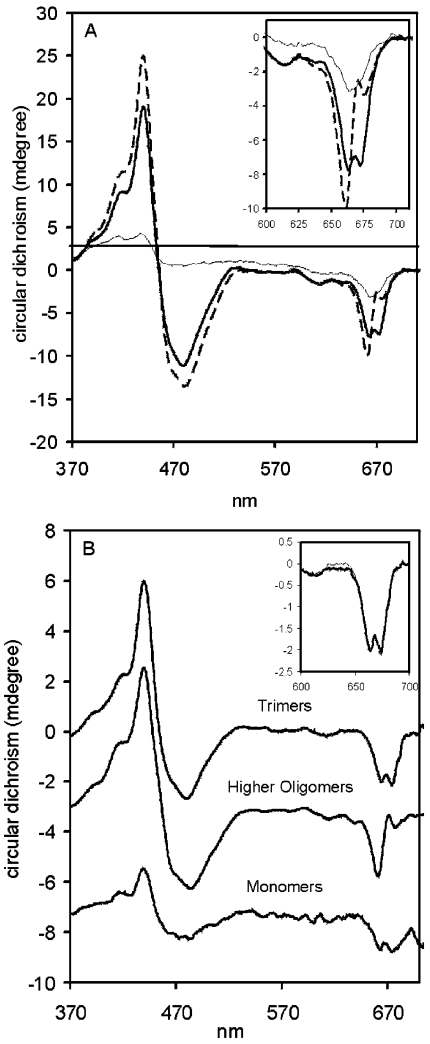

FIGURE 4: Circular dichroism spectra. (A) Spectra of the different fractions harvested from the sucrose gradients, free pigment (thin line), fraction A (bold line), and fraction B (dashed line), respectively. The inset shows an enlargement of the red wavelength region. (B) Spectra of the different oligomeric states isolated by gel filtration. The spectrum of higher oligomers was shifted by −3 mdeg, and the spectrum of monomers was shifted by −7.5 mdeg. The inset shows the similarity between native trimers isolated from fraction A (bold line) and fraction B (thin line) in the red wavelength region.

439.5 nm is indicative of a strong carotenoid−Chl *a* interaction in both gradient fractions. The excitonic interactions of pigments further demonstrate the intactness of the FCPs isolated by this method.

The oligomeric state of the complexes was checked by analytical gel filtration. Fraction A showed elution peaks at two different volumes (column fractions 2 and 3, Figure 5A). Gradient fraction B additionally contained a compound of higher molecular weight (column fraction 1). In both elution profiles, the fraction eluting at 1.3 mL (column fraction 2) was most dominant. Since both gradient fraction A and gradient fraction B contained FCP polypeptides without contaminants, the different fractions on gel filtration must represent different oligomeric states of the FCPs.

To calibrate the gel filtration system, a protein similar to FCPs was used. Monomers and trimers of higher plant LHCII were prepared the same way as FCPs and run under identical conditions on the gel filtration column. From this calibration,

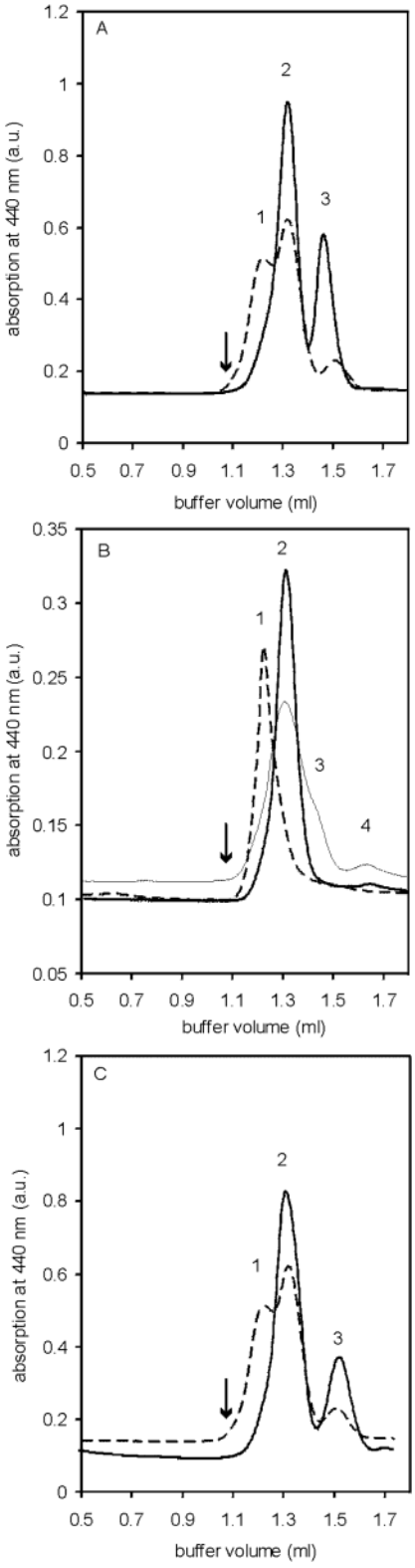

FIGURE 5: Elution profiles measured at 440 nm of different gel filtration experiments. Same numbers denote identical fractions in the different runs. An arrow indicates the void volume of the column. (A) Elution profile of fraction A (solid line) and fraction B (dashed line) harvested from sucrose gradients. (B) Elution profile of trimers (control, bold line) and higher oligomers (dashed line) rerun on the gel filtration system and trimers incubated with phospholipase (thin line). (C) Elution profiles of fraction B harvested directly from the sucrose gradients (dashed line) and after storage at 4 °C for several days (solid line).

it can be concluded that the main fraction found, column fraction 2, consists of trimers. Column fraction 3, which was only prominent in the elution profile of gradient fraction A, represents monomers. Fraction 1 eluted after the void volume of the column and contained functional proteins (see next), so it represents a different, higher oligomeric state. To rule out the possibility of unspecific aggregation due to the low salt conditions during preparation, FCPs were purified using different KCl concentrations. No differences could be found in the range of 2−200 mM KCl. On the basis of areas under the peaks in the elution profiles, the amount of higher oligomers in fraction B was 32−37% at all concentrations used. The same results were obtained when using twice the amount of detergent (20 mM). Additionally, no protein aggregates became visible on negatively stained specimens in the electron microscope (data not shown). Thus, column fraction 1 consists of higher oligomers of the FCPs. The lack of defined higher oligomeric states of LHCII prevented a reliable calibration of the gel filtration column for this fraction. On the basis of calculations using only the molecular weights of monomers and trimers, the higher oligomers consist of six to nine monomers of FCPs.

To confirm the trimeric nature of column fraction 2, it was subjected to nondenaturing gel electrophoresis (Figure 2B, lane 1). It is known from earlier work (*9*, *30*) that the antenna complexes containing Chl *c* are extremely unstable. They lose a significant part of their pigments under all known electrophoretic conditions and are separated mostly as monomers. As expected, a high amount of pigments was removed from the protein and found as free pigment. Only one further pigmented band representing monomers was visible. Therefore, the gel was stained with Coomassie to detect proteins with little pigmentation as well. Two further bands were stained, which appear to be dimers and trimers according to their molecular weight of around 45 and 66 kDa (Figure 2B, lane 1). Thus, even mild electrophoretic conditions led to a stepwise degradation of FCPs, but the experiment confirmed that trimers are the most likely oligomeric state of column fraction 2.

To check whether the different oligomeric states resolved on the gel filtration system were already present in the bands on the sucrose gradients or induced by the column chromatography, column fractions were harvested and rerun under the same conditions. In the case of the trimers, no differences could be found (Figure 5B) (i.e., the gel filtration did not break the trimers into monomers). The same was true for the higher oligomers, which remained intact (Figure 5B). Thus, we conclude that the fractions separated by gel filtration represent the original composition of the sucrose gradient fractions.

The FCPs were separated by equilibrium centrifugation. This raises the question as to why the bands contained different oligomeric states. The monomers in fraction A were more likely due to insufficient separation on the sucrose density gradients than to the breakdown of trimers because the trimers were rather stable. Fraction B consisted of more lipids than fraction A (on the basis of equal amounts of Chl, data not shown). It most likely represented parts of the membrane with loosely bound trimers and higher oligomers together that were not solubilized under the mild conditions that had to be applied to keep the FCPs intact. This raises the question as to whether the trimers of gradient fractions A and B are identical and whether they are associated as higher oligomers in vivo. Therefore, all fractions isolated by gel filtration were checked by SDS−PAGE and spectroscopy.

Spectral and Biochemical Characterization of the Different Oligomeric States. Gradient fraction A was enriched in the 18 kDa polypeptides, and all the fractions it contained showed the same pattern (i.e., trimers (column fraction 2, Figure 2C, lane 1) as well as monomers (column fraction 3)). In the case of gradient fraction B, trimers also contained the 18 kDa polypeptide (Figure 2C, lane 2), whereas the higher oligomers were enriched in the 19 kDa polypeptide (Figure 2C, lane 3). Because trimers were composed of 18 kDa subunits, they could not represent the basic unit of the higher oligomers. On the contrary, the oligomers have to represent a different supramolecular organization preferentially adopted by the 19 kDa subunits.

No disassembly of higher oligomers was induced by rerunning them on the gel filtration column. However, when higher oligomers were stored at 4 °C for several days, they dissociated into trimers and monomers (Figure 5C). Incubating higher oligomers with an increased concentration of detergent (50 mM) led to the same result. This proves that principally two forms of trimers exist: (i) trimers that are mainly composed of polypeptides of 19 kDa and are the basic unit of the higher oligomers and (ii) trimers that are mainly composed of 18 kDa polypeptides and were found in both sucrose gradient fractions. *Fcp1*, *fcp2*, *fcp3*, or *fcp4* encode 18 kDa polypeptides, and the mRNA levels of these proteins are increased in low-light cultures as compared to cells grown under high light conditions (*31*). This explains the dominance of the trimers of 18 kDa subunits found here. Only these trimers are considered when referring to native trimers later on.

Figure 6 shows the absorbance and fluorescence excitation spectra of the different FCPs. The spectra obtained from native trimers of either gradient fractions A or B were very similar confirming their identity. In addition, the spectra of the higher oligomers closely resembled those of native trimers (Figure 6A,C) (i.e., their pigmentation is almost identical). Excitation energy transfer to Chl *a* was efficient in both native trimers and higher oligomers but neither in monomers nor in the trimers that contained 19 kDa polypeptides and were isolated from aged higher oligomers. Their absorbance was more pronounced at 486 nm and less strong at 565 nm when compared to native trimers or higher oligomers (Figure 6B). The increase at 486 nm was not visible in the fluorescence excitation spectra (Figure 6D) and is most likely due to a spectral shift of pigments having lost their proper arrangement. Thus, monomers as well as the trimers containing 19 kDa polypeptides were impaired in excitation energy transfer to Chl *a*.

Figure 4B shows the CD spectra in the visible wavelength range. As for all other data presented here, it has to be concluded that the trimers isolated from gradient fractions A and B are identical. In the Q_y region of Chl *a*, a split band is visible with negative peaks at 663 and 674 nm, separated by a more positive signal at 669 nm and accompanied by a shoulder at around 679 nm. The negative band at 663 nm is much more pronounced in the higher oligomers and seems to be indicative of a higher oligomeric state. Monomers were greatly impaired in their function during the isolation

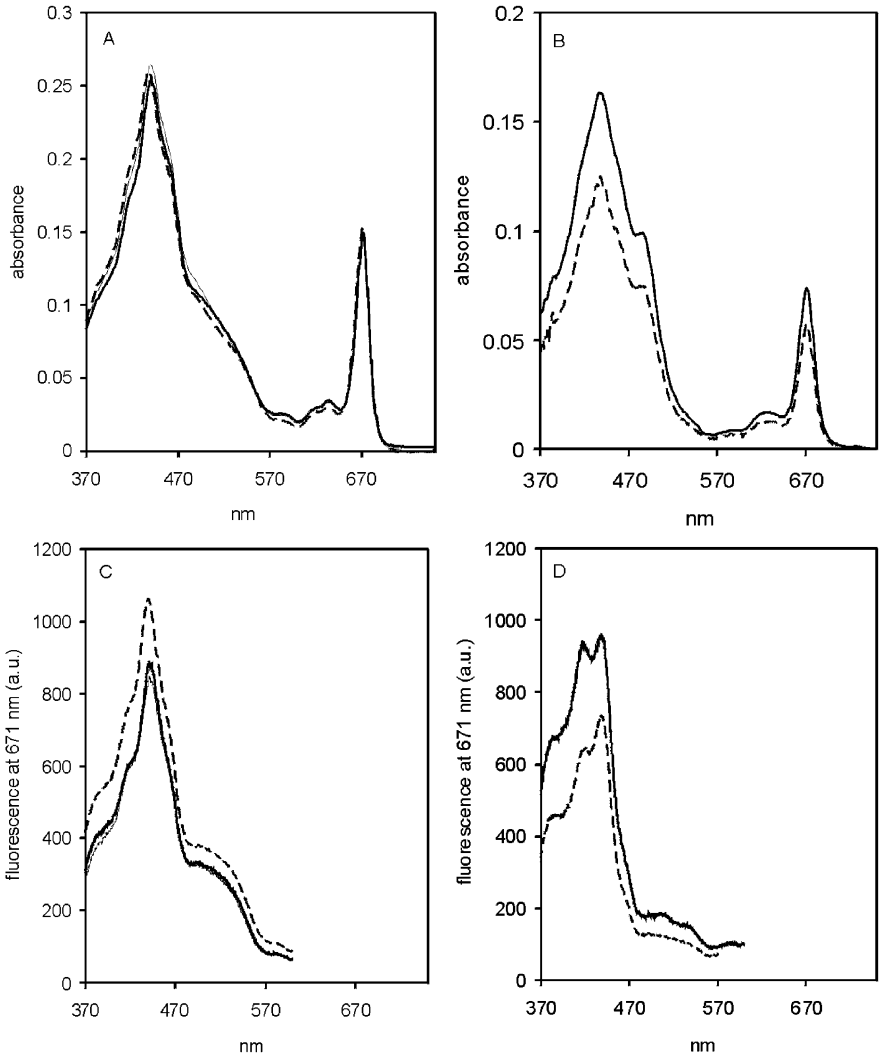

FIGURE 6: Room-temperature spectra of the different oligomeric states of FCPs. (A and B) Absorbance spectra. (C and D) Fluorescence excitation spectra recorded at 671 nm. (A and C) Spectra of native trimers isolated from fraction A (bold line), native trimers of fraction B (thin line), and higher oligomers (dashed line). (B and D) Spectra of monomers (solid line) and spectra of trimers composed of 19 kDa subunits that were isolated from aged oligomers (dashed line). Monomers were measured using half the Chl *a* content as compared to native trimers and higher oligomers (0.75 μg/mL), and trimers consisting of 19 kDa polypeptides were measured at 0.5 μg/mL Chl *a*.

procedure. Therefore, the CD spectra of monomers were inherently noisy, but they principally resembled those of the trimers.

It can be concluded that the native trimers isolated from either band of the sucrose gradients consist mainly of 18 kDa polypeptides and are spectroscopically identical to each other. Fucoxanthin and Chl *c* are properly bound to the polypeptides and transfer absorbed energy to Chl *a*. Thus, the absorbance and fluorescence spectra resembled those from complexes prepared previously from related organisms (*8*, *16*−*19*, *32*). The higher oligomers consist mainly of polypeptides of 19 kDa and are also able to transfer absorbed light energy efficiently to Chl *a*. They are composed of trimers as well, which in contrast to the native trimers containing 18 kDa proteins are not stable as such. Protein assembly of these trimers into higher oligomers is so stable that for disruption rather harsh means have to be applied, which also destroys the capability of energy transfer to Chl *a*. Although different in polypeptide composition, the spectral properties of native trimers and higher oligomers are principally the same with the exception of their circular dichroism in the red wavelength region. Previously, different oligomeric states of FCPs analyzed using CD spectra were only reported from a brown alga, *Dictyota dichotoma* (*17*). Whereas the CD spectra of *D. dichotoma* and *C. meneghiniana* are similar in the blue wavelength region, indicating a strong interaction between fucoxanthin and Chl *a*, there are substantial differences around the Q_y absorption of Chl *a*. In all oligomeric states shown for *D. dichotoma*, there is only one negative CD band at 672 nm, whereas in *C. meneghiniana*, a split signal is present. These traces indicate that at least three different CD-active Chl *a* molecules are bound to the FCPs in *C. meneghiniana*, which interact excitonically. The strength of the bands differed between trimers and higher oligomers whereby the band at the shortest wavelength, 663 nm, is most pronounced in the oligomers. This signal might either indicate a slightly different binding of Chl *a* to the 19 kDa polypeptides as compared to the 18 kDa proteins or be due to interactions between Chl *a* molecules that exist only in the higher oligomeric state. The latter would resemble the situation in higher plants where long-range interactions between LHCII complexes in the

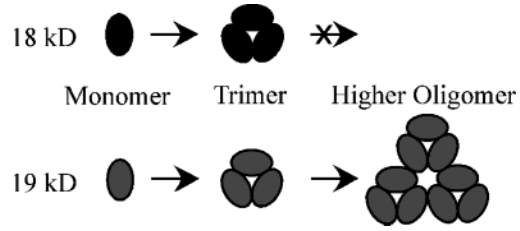

FIGURE 7: Model of the different assemblies of FCPs found in *C. meneghiniana*. Monomers of 18 kDa assemble into trimers, whereas 19 kDa proteins associate via trimers into higher oligomers.

native thylakoid membrane induce a special CD signal. This signal is, however, much stronger than the signals shown here (*33*).

Monomeric FCPs had lost most of the energy coupling between carotenoids and Chls. Part of the monomers might be comparable to the minor LHC of higher plants as speculated before (*11*), but this question will be addressed separately.

Monomerization of Trimers. Trimerization of LHCII is mediated by phosphatidyl glycerol (*34*−*36*), and phospholipids might play a role in the trimerization of FCPs as well. To test this, trimeric FCP was incubated for 20 min with phospholipase A_2. Figure 5B shows that by this treatment, no complete monomerization was achieved. The elution profile indicates unspecific breakdown with a broad peak of trimers, maybe dimers, and monomers. In addition, fraction 4 was detected under these conditions and most likely contained free pigments. To elucidate this problem further, phospholipase treated trimers were run on nondenaturing gels as shown previously (Figure 2B, lane 2). After further disintegration during electrophoresis, no trimers and only a faint dimer band are visible, and the amount of monomers is substantially increased as compared to control trimers. However, phospholipids are certainly not as crucial for trimerization as in LHCII, where the same treatment leads to almost complete dissociation into monomers (*35*).

Conclusions. In diatoms, the grana-stroma differentiation of thylakoid membranes is missing, and in the related brown algae, all proteins visualized using freeze-fracturing are more evenly distributed as compared to higher plant thylakoids (*11*, *37*). Here, we report on different oligomeric states of the FCP proteins. FCPs, which from their sequence are predicted to adopt a similar tertiary structure as LHCII (*38*), are also able to form trimers. In contrast to higher plants, where phosphatidyl glycerol is the decisive agent for trimerization (*34*−*36*), these trimers seem to be formed by different interactions. The thylakoid membrane of *C. meneghiniana* contains trimers that are mainly composed of 18 kDa subunits. In addition, higher oligomers are present, which most probably represent hexamers or nonamers (Figure 7). Higher oligomers are built of trimers that contain mainly 19 kDa subunits. The protein−protein interaction between trimers in the higher oligomers is rather tight since all methods to disassemble the higher oligomers disrupt excitation energy transfer to Chl *a*. This assembly of special subunits of antenna proteins into higher oligomeric states is a feature not known from higher plant LHCs. In accordance with the lack of grana-stroma differentiation of thylakoid membranes, the organization of antenna proteins in diatoms differs with respect to the oligomeric states that the different FCP proteins can adopt.

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