

# Spatial arrangement of pigments and their interaction in the fucoxanthin-chlorophyll *a/c* protein assembly (FCPA) isolated from the brown alga *Dictyota dichotoma*. Analysis by means of polarized spectroscopy

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Molecular interaction and energy transfer between pigments in the fucoxanthin-chlorophyll (Chl) *a/c* protein assembly (FCPA) isolated from the brown alga *Dictyota dichotoma* was investigated mainly by polarized spectroscopy. FCPA consists of 7 identical units of 54 kDa apoprotein, each of them containing 13 Chl *a*, 3 Chl *c*, 10 fucoxanthin and 1 violaxanthin. Spectral heterogeneity was found in the component pigments; two types of Chl *a* (Chl *a*<sub>673</sub> and Chl *a*<sub>669</sub>), two types of Chl *c* (Chl *c*<sub>1</sub>, long-wavelength form of Chl *c* and Chl *c*<sub>s</sub>, short-wavelength form). In fucoxanthin, two functionally active and one inactive species were found. Energy flow in the FCPA is attained by a direct coupling of donor-acceptor pair and those are classified into four types: from fucoxanthin to Chl *a*<sub>669</sub>, from fucoxanthin to Chl *a*<sub>673</sub>, from Chl *c*<sub>1</sub> to Chl *a*<sub>669</sub> and from Chl *c*<sub>s</sub> to Chl *a*<sub>669</sub>. The number of those four pathways was estimated to be 7, 2, 2 and 1, respectively, per unit peptide. Energy migration in the Chl *a* molecules is always functioning. Dissociation of FCPA into unit peptides induces the uncoupling of energy transfer between the respective donor and acceptor Chl *a*. The spatial orientation of individual pigments, investigated by linear dichroism and polarized fluorescence spectroscopy, was shown to be favorable for an efficient energy transfer. Based on the results of polarized spectroscopy, a spatial orientation of individual chromophores in the peptide was proposed.

## Introduction

We have recently isolated a supramolecular assembly of light-harvesting pigment proteins from the brown alga *Dictyota dichotoma* using a new type of mild detergent, decyl sucrose [1]. The pigment protein complexes are in an assembly as a functional form, in contrast to any other preparations of Chl *a/c*-carotenoid proteins so far studied [2,3]. This complex is composed of several (most probably 7) identical units, each of them containing Chl *a*, Chl *c*, fucoxanthin and violaxanthin in the molar ratio of 13:3:10:1 in 54 kDa apoprotein [1]. Hence, the assembled form is called 'fucoxanthin-Chl *a/c* protein assembly' (FCPA) for the

functional form of the pigment-protein complex. In this assembled form, a highly efficient energy transfer was observed from fucoxanthin and Chl *c* to Chl *a*, which leads to the idea that the FCPA is a native form.

The mechanism for the energy transfer from carotenoid to chlorophyll in photosynthetic pigment protein complex has as yet not been clearly elucidated (cf. Ref. 4). An electron exchange mechanism is proposed for this transfer process [5], contrary to the Förster mechanism as proposed for the transfer between chlorophyll molecules or between phycobiliproteins [6]. The exchange mechanism requires a close location of the donor-acceptor pair and suitable mutual orientation, which gives rise to overlap of the electron cloud. Therefore, topology of the pigments in the complex is the primary factor in determining the transfer efficiency.

The molecular topology of the carotenoid-Chl complex has been well studied in the case of peridinin-Chl *a* protein complex isolated from the marine dinoflagellate, *Glenodinium* sp [7]. Four peridinin molecules and one Chl *a* are associated with the apoprotein in a molecular

Abbreviations: CD, circular dichroism; Chl, chlorophyll; FCPA, fucoxanthin-chlorophyll *a/c* protein assembly; LD, linear dichroism.

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mass of about 39 kDa [8,9], and an exciton interaction between peridinin molecules has been clearly shown by circular dichroism (CD) spectroscopy [7]. The dipole moment of the peridinin is estimated to be about  $45^\circ$  to that of the  $Q_y$  transition of the Chl *a* molecules; however, no strong interaction between peridinin and Chl *a* was observed. The peridinin-Chl *a* protein is a so-called water-soluble protein, leading to a quite simple isolation method and consequent extensive investigations under conditions without the effect of detergent [7]. This is not the case for the membrane-bound fucoxanthin-Chl protein complex [1,2].

In this paper, we have investigated the topology and molecular interaction between pigments in the FCPA isolated from the brown alga *D. dichotoma*. Several kinds of polarized spectroscopy were applied to these pigment-protein complexes to analyze their optical properties and their changes due to the association state. No strong interaction between fucoxanthins or fucoxanthin and chlorophylls was observed. However, one-to-one coupling between fucoxanthin and Chl *a* and a spatial orientation of pigment molecules was elucidated, both of which are favorable for an efficient energy transfer between pigments in the FCPA.

## Materials and Methods

**Isolation of pigment protein complex.** FCPA was isolated from the brown alga *D. dichotoma* by a newly developed method [1]. Thalli were collected from the intertidal zone of the Oshoro-bay, Hokkaido area, Japan, frozen until use. FCPA was extracted from the French-pressed thalli using 1% decyl sucrose (Mitsubishi-Kasei Foods Co., Tokyo, Japan) and purified by sucrose density gradient. Monomeric form was obtained either by dilution of protein concentration or addition of Triton X-100 (final 0.02%).

**Isolation of membrane fragments.** After passing the French-press, broken thalli were subjected to sonication for 1 min (six times of 10 s sonication) at  $2^\circ\text{C}$ . Debris was removed by centrifugation at  $5000 \times g$ , 10 min.  $\text{MgCl}_2$  (2 mM) was added to the supernatant and stood for 10 min at  $2^\circ\text{C}$ . Membrane fragments were collected by centrifugation at  $37000 \times g$  for 60 min.

**Spectroscopic analysis.** Absorption and CD spectra at room temperature were measured with a Hitachi 330 spectrophotometer and a Jasco J-200B spectropolarimeter, respectively. Fluorescence and polarized fluorescence were measured with a Hitachi 850 spectrofluorometer with glass plate polarizers. Linear dichroism (LD) was measured by the gel-squeezing method. Samples were embedded in polyacrylamide gel (12%) containing 30% sucrose and 100 mM potassium phosphate buffer (pH 7.0) (cf. Ref. 10) and the gel was uniaxially stretched. Spectra were measured on samples with half the original thickness using a Jasco J-200B

spectropolarimeter equipped for LD measurement at  $23^\circ\text{C}$ . Absorption spectra of the gel before stretching were separately measured with a Hitachi 330 spectrophotometer. For measurements of fluorescence spectra at  $-196^\circ\text{C}$ , samples were mixed with an equal volume of 30% poly(ethyleneglycol) 4000 to obtain homogeneous ice, and then immersed into liquid nitrogen during measurements.

**Data analysis.** All the spectroscopic data were transferred to a micro-computer (HP model 216) and numerically analyzed. For improving the signal-to-noise ratio, spectra were measured several times and added together. Fluorescence and excitation spectra were corrected based on the radiation profile of a standard tungsten lamp whose color temperature was known. Correction factors for fluorescence polarization spectrum were obtained by the method of Azumi and McGlynn [11]. Deconvolution of spectra was carried out based on the least-square method [12,13].

## Results

In our previous study [1], we have reported spectral heterogeneity of fucoxanthin and Chl *c*, based mainly on the second-derivative spectrum (Fig. 2 in Ref. 1). Heterogeneity of components suggests the occurrence of plural energy transfer pathways. So we intensively investigated this point at first by various kinds of polarized spectroscopy.

### *Heterogeneity of Chl a detected by CD spectroscopy*

Three CD bands were observed in the spectrum of associated forms, i.e., FCPA (Fig. 1B): the 673 nm (–), 475 nm (–) and 440 nm (+) bands (signs in the parentheses represent the sign of the CD signal). The first and last bands correspond to the  $Q_y$  and Soret bands of Chl *a*, and the second band to a specific form of carotenoid, most probably fucoxanthin. Compared with the absorption spectrum (Fig. 1A), the maximum of  $Q_y$  band of Chl *a* was shifted to the red by 2 nm (673 nm), indicating heterogeneity of Chl *a*, one component being a chiral and red-shifted form. This form corresponds to the 673 nm absorption band found by the difference absorption spectrum [1]. A simple negative peak at 673 nm indicates that strong interaction between Chl *a* molecules is absent from the FCPA. The Chl *c* CD signal was not detected in either the  $Q_y$  or the Soret region (around 460 nm). A distinct CD band was observed around 475 nm, corresponding to fucoxanthin. The excitation spectrum for the Chl *a* emission (Fig. 4 in Ref. 1) revealed that the absorption maximum of the functional form of fucoxanthin is located around 510 nm. The observed CD band does not coincide with this functional component, indicating the presence of at least two spectral forms in fucoxanthin.

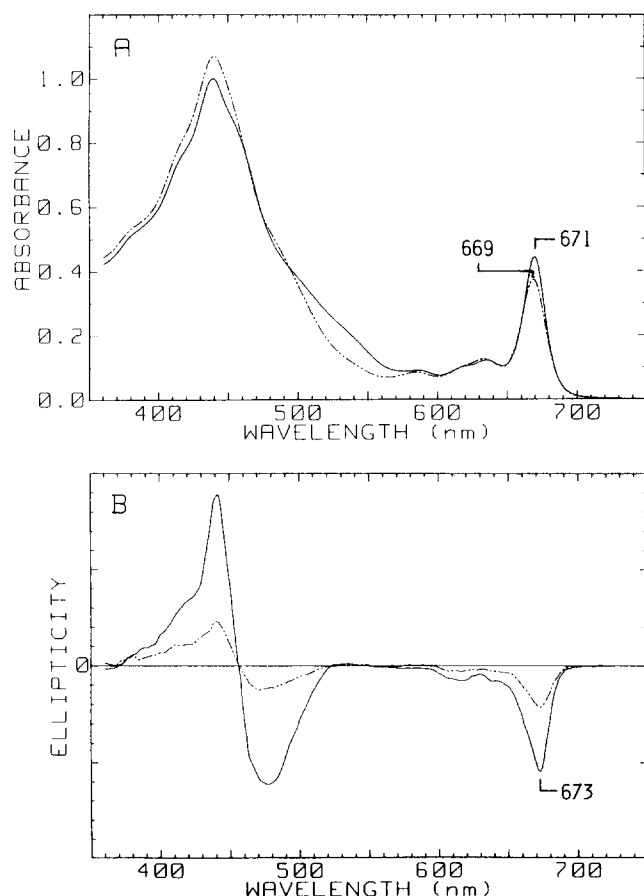


Fig. 1. Absorption (A) and CD (B) spectra of FCPA at room temperature. In both figures: —, the spectra in the assembled form and ---, in the dissociated form. For details, see the text.

Upon dissociation of FCPA into monomers, changes in the CD spectrum were small (Fig. 1B). The maximum of Chl *a* was still located at 673 nm; but a decrease in the intensity by about 60% was observed. This was also the case for the carotenoid region, as evidenced by the same zero-crossing point. In the absorption spectrum, a clear blue shift of fucoxanthin was observed (Fig. 1A); however, no corresponding change was observed in the CD spectrum. Dissociation of FCPA into monomer might induce changes in the polypeptide structure which consequently give rise to the spectral changes in the carotenoids.

#### Presence of two transfer pathways from Chl *c*

The heterogeneity of Chl *c* has been evidenced by plural Soret bands in the absorption spectrum [1]. Corresponding plural  $Q_y$  bands were found by the second derivative spectrum at  $-196^\circ\text{C}$  [1]. Furthermore, two transfer pathways from Chl *c* were found at  $-196^\circ\text{C}$  (Fig. 2A). When the FCPA was excited at 450 nm, the emission of Chl *c* was observed at 633 nm besides the main band at 675 nm, whereas when excited at 465 nm, the Chl *c* emission shifted to 638 nm. These results

indicate the absence of energy transfer between Chl *c* molecules. The excitation spectrum for the respective emission maximum was different (Fig. 2B), though the difference was not so prominent due to an overlap between two emission bands. The contribution of the 450 nm region was higher for the 633 nm emission, and also for the case of the band around 465 nm, to the 638 nm emission. The corresponding  $Q_y$  band of the 450 nm component is expected to be located at a shorter wavelength than that of the 465 nm component. In the absorption spectrum at  $-196^\circ\text{C}$  (Fig. 2B inset), there were two maxima corresponding to Chl *c* (632 and 638 nm). Thus is reasonable to conclude that there are two types of Chl *c* molecule: a short-wavelength form (abbreviated to Chl  $c_s$ ), and a long-wavelength form (Chl  $c_l$ ), each of them transferring energy to Chl *a*. At this

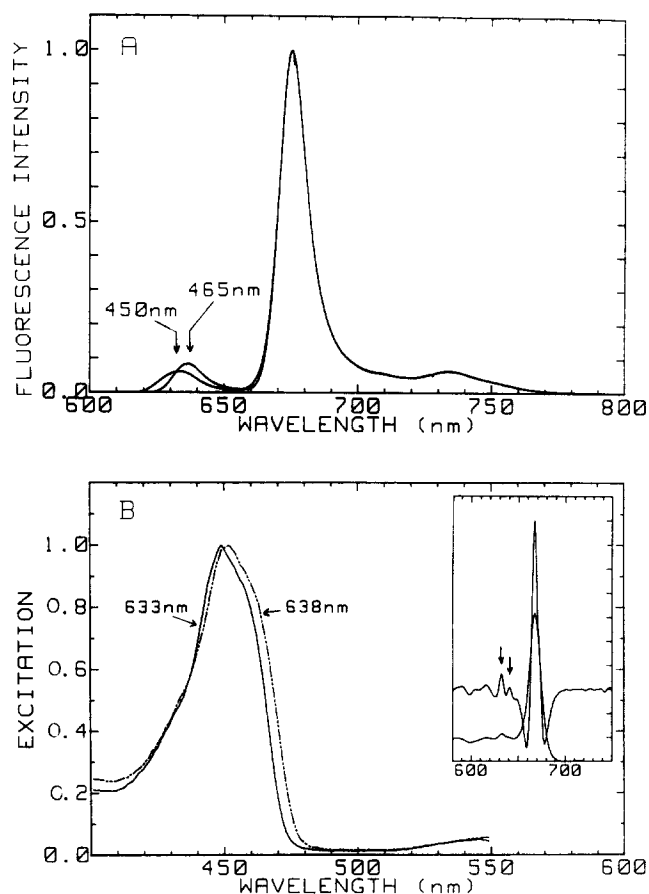


Fig. 2. Fluorescence spectra (A) and excitation spectra (B) of FCPA at  $-196^\circ\text{C}$ . FCPA was suspended in the buffer containing 15% poly(ethyleneglycol) 4000. In (A), excitation wavelengths were 450 and 465 nm, respectively, for the left or right spectrum in the region around 630 nm. Arrows indicate the locations of the maxima. As for (B), excitation spectra were monitored at 633 (—) and 638 (---) nm, respectively. Spectra were normalized at their maxima. Inset: absorption and second derivative spectra of FCPA at  $-196^\circ\text{C}$ . The second derivative spectrum was shown after inversion. Arrows show the locations of Chl *c* maxima in the second-derivative spectrum. For details, see the text.

experimental stage, it is not clear whether the presence of two forms is due to differences in the molecular species (Chl  $c_1$  and/or Chl  $c_2$ ) or due to the environmental factors in the polypeptides.

#### Deconvolution of absorption spectrum into components

The absorption spectra of the two different association states were resolved into component spectra by deconvolution (Fig. 3). A Gaussian band shape was assumed, because the absorption spectrum of Chl  $a$  in organic solvents was well reproduced by the sum of such types of band [13]. Based on the difference and second-derivative spectra (cf. Fig. 2 in Ref. 1), two Chl  $a$  and two Chl  $c$  bands were assumed, and deconvolution was carried out on the spectra measured at room temperature. As for the Chl  $a$  form, the 673 and 669 nm components were resolved. The location of the latter was slightly shorter than the estimation by the second-derivative spectrum (670 nm, Fig. 3A). These two forms

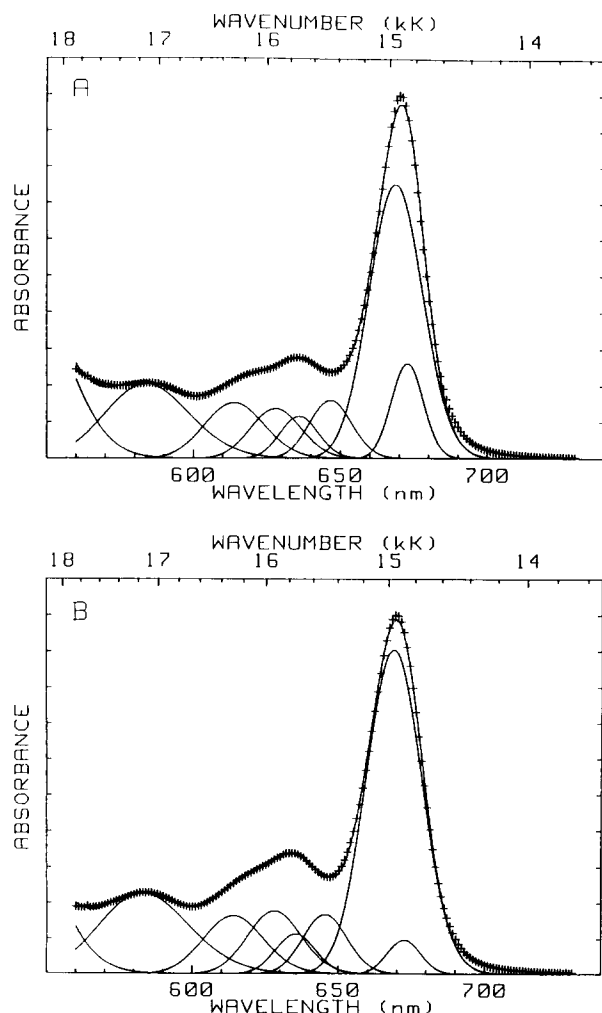


Fig. 3. Deconvolution of absorption spectra in the assembled form (FCPA) (A) and dissociated unit peptides (B) at room temperature. The spectrum was assumed to be the sum of component bands with a Gaussian band shape. For details, see the text.

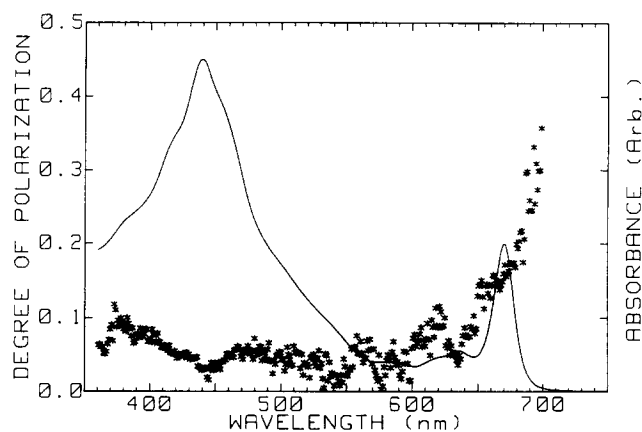


Fig. 4. Fluorescence excitation polarization spectrum of FCPA at room temperature. Fluorescence was monitored at 710 nm. Half-bandwidth for excitation was 3 nm and for the emission, 2 nm. The absorption spectrum of the assembled form is also shown. For details, see the text.

are hereafter called Chl  $a_{673}$  and Chl  $a_{669}$ , respectively. The molar ratio of the former to the latter was about 0.18, based on the integrated area in wavenumber unit under each absorption band. Since the total number of Chls  $a$  in unit peptide was 13 [1], the above ratio indicates the actual number of molecules to be 3 and 10 for Chl  $a_{673}$  and Chl  $a_{669}$ , respectively, in the FCPA. When the FCPA was dissociated into monomers (Fig. 3B), the ratio of Chl  $a_{673}$  to Chl  $a_{669}$  became 1 to 12, indicating that two of the Chls  $a_{673}$  turn into the Chl  $a_{669}$ . This change in the number agrees well with changes in the CD intensity (decrease by about 60%, Fig. 1B). On Chl  $c$ , two bands were resolved, at 628 and 636 nm. The molar ratio of the shorter component (Chl  $c_s$ ) was always higher than that of Chl  $c_l$ . The estimated molar ratio of Chl  $c_s$  to Chl  $c_l$  was 2. In FCPA, three Chl  $c$  molecules are present, and thus it can be reasonably concluded that two of them correspond to the Chl  $c_s$  and the remaining one to the Chl  $c_l$ . The component band at 645 nm is a vibrational band, as usually found in the spectra of Chl  $a$  in organic solvents (cf. Ref. 13).

#### Fluorescence excitation polarization spectrum

Some characteristic aspects of absorption were also monitored by fluorescence polarization spectra. As shown previously [1], the main emission band of FCPA at room temperature was located at 677 nm. For the detection of characteristics of Chl  $a$ , fluorescence should be monitored at longer wavelength. Thus, the monitoring wavelength was set to 710 nm to examine the coupling between pigments in the FCPA (Fig. 4).

One feature is the heterogeneity of Chl  $a$  as evidenced by a remarkable increase in the degree of polarization up to 0.37 only at the red edge of the absorption spectrum. This clearly indicates the presence of two types of Chl  $a$ , one of which is the terminal emitter. Two absorption components with similar band shapes

but in slightly different locations will usually give this kind of pattern. The shorter wavelength component is a sensitizer, most probably corresponding to the Chl  $a_{669}$  and the longer, a fluorescing component, corresponds to the Chl  $a_{673}$ .

The second feature is the contribution of Chl  $c$ . As seen in the spectrum (Fig. 4), the degree of polarization in the wavelength region for Chl  $c$  (around 630 nm) is evidently lower than that of Chl  $a$  (both in  $Q_{y0-0}$  and  $Q_{x0-0}$ ), but higher than that of fucoxanthin. This indicates that Chl  $c$  is not the intermediate of energy transfer from fucoxanthin to Chl  $a$  via the  $Q_x$  band, as observed by the absence of Chl  $c$  emission under conditions of fucoxanthin excitation (cf. Fig. 3 in Ref. 1).

Thirdly, the degree of polarization in the region for fucoxanthin was lower than that of the  $Q_y$  band of Chl  $a$  but almost the same as that of the  $Q_x$  band of Chl  $a$ . If the  $Q_x$  band of Chl  $a$  plays an essential role in the energy transfer from fucoxanthin to Chl  $a$ , the dipole moments of both pigments can be assumed to be nearly parallel to each other, as suggested in the case of the bacterial antenna system [14].

Based on the degree of polarization, the mutual orientation of dipole moment of respective pigments can be calculated. The average value of the degree of polarization for the red edge of the absorption was 0.292. The values for respective pigments were estimated to be 0.146, 0.043 and 0.038 for Chl  $a$ , Chl  $c$  and fucoxanthin, respectively. These values should be regarded as average, because the absorption bands overlap each other, which makes the estimation of individual pigments difficult. These values gave the mutual angles of individual dipole moments:  $36^\circ$  between Chl  $a_{673}$  and Chl  $a_{669}$ ,  $43^\circ$  between Chl  $c$  and the  $Q_y$  band of Chl  $a_{669}$ ; and  $45^\circ$  between fucoxanthin and the  $Q_y$  band of Chl  $a_{669}$ . The difference in the degree of polarization between fucoxanthin and the  $Q_x$  band of Chl  $a$  was very small, indicating them to be nearly parallel to each other.

As stated above, energy transfer from Chl  $c$  to Chl  $a$  was direct. This seems to be the case in fucoxanthin and Chl  $a$ . The transfer step between these two pigments might be only one, because if this is not the case, the level of the degree of polarization of fucoxanthin should be much smaller, close to zero. This suggests direct interaction between Chl  $a$  and fucoxanthin in this complex; however, the possibility of the energy transfer between fucoxanthin molecules oriented in parallel cannot be ruled out.

#### LD spectrum

The direction of the dipole moment can also be observed by the LD spectrum ( $\Delta A = A_{\parallel} - A_{\perp}$ , Fig. 5A). The absorption spectrum of FCPA in 12% gel was almost the same as in the buffer solution, but with a minor difference in the shape of the Soret band of Chl

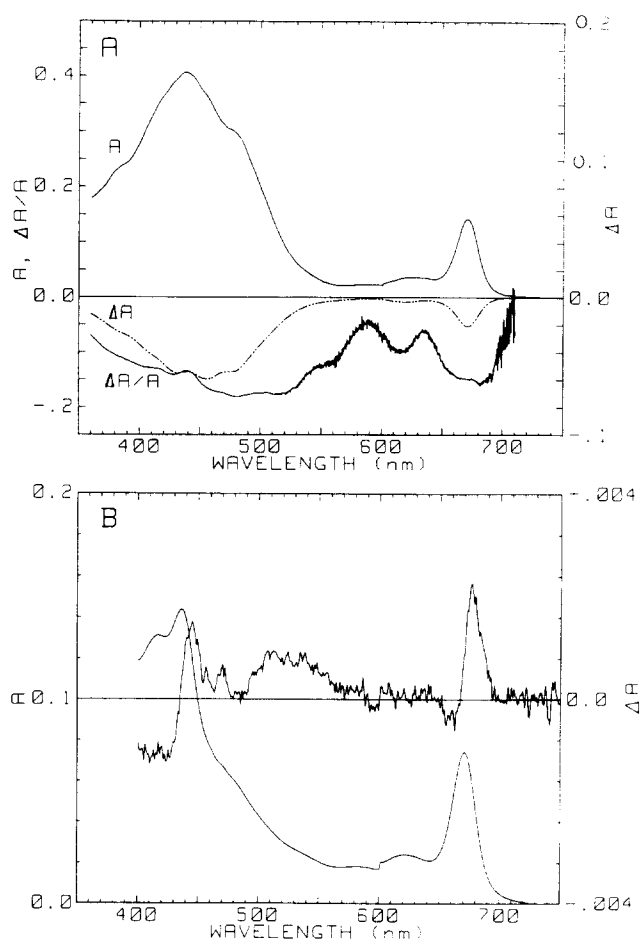


Fig. 5. LD spectra of FCPA (A) and of membrane fragments (B) at room temperature. Samples were embedded in 12% polyacrylamide gel and uniaxially stretched. In (B), the spectrum in the wavelength region shorter than 400 nm is not shown because of the scattering effect. For details, see the text.

*a*. The LD spectrum obtained by uniaxially stretched sample showed a characteristic negative bands, indicating a dipole moment quite perpendicular to the orientation axis. The spectrum of Chl  $a$  was very similar to the absorption spectrum; in contrast, that of the carotenoid was different: three clear maxima at 428, 455 and 480 nm, similar to the typical absorption spectrum of carotenoid in organic solvents [15]. Again, no peak for Chl  $c$  was detected in the LD spectrum, indicating that the orientation of the dipole moment of Chl  $c$  lies close to the magic angle to the stretching axis.

Heterogeneity of the component bands was revealed by the reduced LD, that is,  $\Delta A/A$  (Fig. 5A). Two bands were resolved in the Chl  $a$  region with maxima around 667 and 680 nm. These two might correspond to Chl  $a_{669}$  and Chl  $a_{673}$ , respectively, though the locations of the bands were different from those in absorption spectrum. A clear but smaller peak around 635 nm corresponds to Chl  $c$ , having an orientation different from that of Chl  $a$ . In the carotenoid wavelength region several peaks were resolved. Those can be classified into

two categories; one comprises those bands corresponding to the LD spectrum with three peaks, and the other a relevant peak detected by the reduced LD, that is, bands located around 518 and 555 nm. This classification corresponds well to the function: the former is not active in energy transfer, unlike the latter. The reduced LD value is a function of the projected angle between the orientation axis and the direction of the dipole moment. The value of the reduced LD in the wavelength region of carotenoid is similar to that of the  $Q_y$  band of Chl *a*. This clearly indicates that these two dipole moments are oriented nearly parallel to each other. On the other hand, as for the  $Q_x$  band of Chl *a*, a significant difference (about  $5^\circ$ ) from the dipole moment of fucoxanthin could be estimated.

The LD spectrum of membrane fragments was very different from that of the isolated samples (Fig. 5B). The sign of the Chl *a* band was positive, as has been seen in thylakoid membranes isolated from spinach chloroplasts [16], but opposite to the case of FCPA. Fucoxanthin showed the same orientation as Chl *a*; positive in membrane fragments and negative in the isolated form. These results clearly show that the mutual orientation of both pigments was the same; however, due to the difference in the orientation axis, the apparent spectrum gave the opposite sign in the respective spectra.

## Discussion

Four kinds of pigment are present in the FCPA: Chl *a*, Chl *c*, fucoxanthin and violaxanthin in the molar ratio of 13:3:10:1 in 54 kDa apoprotein [1]. Spectral heterogeneity was found in three of them: Chl *a*, Chl *c* and fucoxanthin. Differentiation of these spectral forms can essentially affect the energy transfer processes in this assembly. Therefore, the molecular assembly of the component proteins and molecular interaction of pigments should be primarily considered, as for the function of FCPA.

### Molecular assembly of FCPA in the membranes

The LD spectrum of membrane fragments (Fig. 5B) indicates that the porphyrin ring of Chl *a* is located about  $30^\circ$  to the membrane plane, as in the case of thylakoid membranes in higher plants [16]. The angle between carotenoids and Chl *a* is small. On the other hand, the orientation of Chl *a* in FCPA was quite perpendicular to the stretching axis (Fig. 5A). These results can be explained by changes in the orientation axis due to isolation. One necessity is the assumption of oblong-shaped FCPA. The orientation axis of the oblong-shaped peptides in the squeezed gel is most probably along the long axis. A negative LD signal of the FCPA indicates that the dipole moments of the Chl *a* are at more than  $55^\circ$  to the orientation axis. When the

FCPA is located in the membranes with its long axis perpendicular to the membrane plane, this arrangement will give a positive LD signal in membranes. This is in good agreement with the observation.

FCPA shows hydrophobic properties, like other integral proteins; however, it is quite selectively extracted by a very mild detergent, decyl sucrose [1]. FCPA is most probably buried in the membranes; however, its location should be investigated for an analysis of overall energy flow.

### Molecular interaction

In the FCPA, there are two types of one-to-one coupling between pigments: one is between Chl *c* and Chl *a*, and the other between fucoxanthin and Chl *a*. Chl *c* does not interact with fucoxanthin. When FCPA was dissociated into component peptides, two molecules of Chl  $a_{673}$  out of three turn into Chl  $a_{669}$ , concomitant with the disruption of energy transfer from Chl *c* and fucoxanthin to Chl *a*. These results suggest two possibilities for the counterpart of the interaction with Chl  $a_{673}$ : Chl *c* or fucoxanthin. Changes in the action spectra in the course of dissociation (Fig. 6 in Ref. 1) clearly indicate that coupling between fucoxanthin and Chl *a* is disrupted first; even later on in dissociation, energy transfer from Chl *c* to Chl *a* was still functioning. These results indicate that out of three Chls  $a_{673}$ , two tightly couple with fucoxanthin and that three Chl *c* molecules interact with respective Chl  $a_{669}$  molecules.

One Chl  $a_{673}$  is always functional: even in the dissociated form, fluorescence maximum was located at 677 nm, as in the case of an assembled form. This most probably originates from Chl  $a_{673}$ . Since this molecule was not affected by dissociation, it might be in a position not affected from the surface (this Chl  $a_{673}$  is

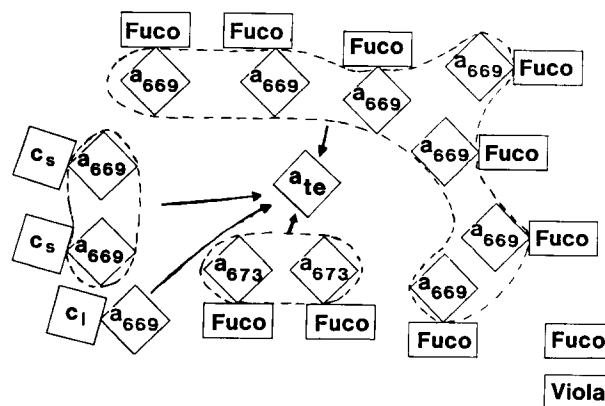


Fig. 6. Schematic representation of energy flow in FCPA unit peptide. The  $a_{669}$  and  $a_{673}$  in squares stand for Chl  $a_{669}$  and Chl  $a_{673}$ , and Fuco and Viola, fucoxanthin and violaxanthin, respectively. The absorption maximum of the Chl  $a_{673}$  was located at 673 nm. The  $c_s$  and  $c_i$  indicate the short-wavelength and long-wavelength forms of Chl *c*. Dashed lines indicate the virtual chlorophyll domains in which the excitation energy can migrate.

designated Chl  $a_{te}$ ,  $te$  meaning terminal emitter, see Fig. 6). Out of 13 molecules of Chl  $a$  in unit peptide, three of them interact with Chl  $c$ , two with fucoxanthin, and one is independent (Chl  $a_{te}$ ). Thus, at most seven molecules of fucoxanthin can couple with remaining seven Chl  $a_{669}$  molecules. The above estimation inevitably yields two uncoupled carotenoids, one violaxanthin and one fucoxanthin. When the action spectrum of Chl  $a$  fluorescence is compared with the absorption spectrum, a significant decrease in transfer efficiency around 480 nm was observed even in the FCPA (cf. Fig. 4 in Ref. 1). Its magnitude is between 15 and 25%, depending on the preparations. Two uncoupled molecules out of 11 will give a decrease in efficiency by 18% with the assumption of 100% efficiency for the transfer from carotenoid to Chl  $a$ . This estimation is in good agreement with the observation. Our estimation is still in a preliminary stage; however, this stoichiometry explains very well the optical properties of the FCPA. In the functional form of fucoxanthin, several spectral forms are present, as shown by the second-derivative spectrum at room temperature (cf. Fig. 2 in Ref. 1). This difference might arise from the difference in the acceptor, Chl  $a_{669}$  and Chl  $a_{673}$ .

#### Energy transfer in FCPA

The energy transfer pathways should be considered for the next step (Fig. 6). There are four types of energy flow to Chl  $a$ ; from Chl  $c_1$  to Chl  $a_{669}$ , from Chl  $c_s$  to Chl  $a_{669}$ , from fucoxanthin to Chl  $a_{669}$  and from fucoxanthin to Chl  $a_{673}$ . One additional Chl  $a_{673}$  (Chl  $a_{te}$ ) is also present. In the FCPA, the fluorescence was always detected at 677 nm, irrespective of the excitation conditions. This indicates that energy is finally transferred to the Chl  $a_{673}$  or Chl  $a_{te}$  through Chl  $a_{669}$ . Energy migration among Chls  $a_{669}$  is possible, but is not yet definitive. When dissociated, the energy transfer between individual pigments and respective acceptor Chls  $a$  is disrupted. The coupling between Chl  $a_{669}$  and Chl  $a_{673}$  (or Chl  $a_{te}$ ) still functions, even after dissociation, except for a shift of Chl  $a_{673}$  to Chl  $a_{669}$ , as evidenced by the absence of fluorescence from Chl  $a_{669}$ .

Two uncoupled carotenoids (one fucoxanthin and one violaxanthin) might have some function in assembly, similar to the case of xanthophylls in the assembly process of LHC II in higher plants [17]. However, their definite function is not clear at this experimental stage.

In the assembled form, energy transfer between monomers should occur. The Chl  $a_{673}$ , sensitive to dissociation and thus presumably located near the

surface of the unit protein, may play a role for the energy transfer under such a condition. For the vectorial energy flow to the core complex, an additional (proteinaceous) component might be present, which facilitates formation of energy gradients and consequently of directional flow to the reaction center.

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