Characterization of Fucoxanthin Aggregates in Mesopores of Silica Gel: Electronic Absorption and Circular Dichroism Spectroscopies

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Abstract

Fucoxanthin shows a large bathochromic shift of its absorption band when it is bound to photosynthetic antenna pigment-protein complexes derived in brown algae (fucoxanthin-chlorophyll *a/c* binding protein; FCP). In order to try to understand the intermolecular interactions of fucoxanthin responsible for this shift, we attempted to create a system that can control intermolecular interactions of carotenoids. In this study, we prepared the fucoxanthin-silica gel (CARiACT Q-6, mesoporous material) conjugates by adsorbing fucoxanthin molecules into the mesopores of CARiACT, and investigated their spectroscopic properties by recording electronic absorption and circular dichroism spectra. Fucoxanthin in the pores of CARiACT showed spectra that suggested the formation of H- or J-aggregates. The form of the aggregate could be controlled by adjusting the ratio of fucoxanthin to CARiACT. The exciton chirality of H-aggregated fucoxanthin was detected by circular dichroism spectroscopy. Based on the point-dipole approximation we were able to determine the molecular arrangement of H-aggregated fucoxanthin in the pores of CARiACT.

Keywords: Carotenoid; Fucoxanthin; Aggregate; Chirality; Mesoporous silica;

1. Introduction

In natural photosynthetic systems, one of the roles of carotenoids is light harvesting: carotenoids absorb light energy and transfer it to chlorophylls [1-3]. Fucoxanthin is a carotenoid bound to the major photosynthetic antenna of brown algae, called the fucoxanthin-chlorophyll *a/c* binding protein (FCP) [4, 5]. The major absorption band of fucoxanthin shows a large bathochromic shift (~50 nm) when fucoxanthin is bound to the FCP. Consequently, the fucoxanthin in FCP can efficiently absorb the blue-green region of the solar spectrum, which is the only part of that spectrum that is available in their habitat several meters below the surface of the sea. The large bathochromic shift could not be simply explained by the polarizability of its environment in FCP; consequently we are trying to investigate whether dipole-dipole interactions from nearby fucoxanthin molecules are also important. However, the key factors that could determine such dipole-dipole interactions, such as distance and orientation between fucoxanthin molecules in the FCP, have not yet been clarified. Therefore, we aimed to create an artificial model system in which we can observe and control their intermolecular interactions in order to try to reproduce the large bathochromic shift.

Molecular aggregates are one of the situations where interactions are present between carotenoid molecules. It is known that excitonic interaction occurs between aggregated

molecules, and their associations in these aggregates are classified into H- (card pack) type and J- (head-to-tail) type, which show a hypsochromic (blue) shift and a bathochromic (red) shift of the absorption band, respectively [6, 7]. Further, such electronic interactions of two individual molecules are clearly observed even if they are separated by as much as 12 nm [8]. Self-aggregation of carotenoid often takes place in aqueous organic solutions [9-15]. Carotenoid aggregates have also been observed in thin solid films [16-18] and in composite molecules [19, 20]. Nevertheless, the self-assembly of fucoxanthin has not yet been reported. Therefore, in the present study, we tried to see if we could observe fucoxanthin aggregates in mesopores materials.

Mesoporous silica is a form of silicon dioxide that has nano-scale pores [21]. Itoh *et al.* reported that the mesopore spaces of FSM (folded-sheet mesoporous material) allowed nano-scale interactions between adsorbed chlorophyll *a* molecules [22-24]. Therefore we attempted to adsorb fucoxanthin molecules into the mesopores of a silica gel called CARiACT and to observe the change of optical properties of fucoxanthin that this produced. In this study, we found we could control the types of aggregates of fucoxanthin by adjusting the concentration of fucoxanthin in solution added to CARiACT.

2. Materials and Methods

2.1. Materials

Special grade benzene was purchased from Kishida Chemical Company, Ltd., Japan. Fucoxanthin was a gift from South Product Company, Ltd., Japan. Both of them were used as received. The silica gel (CARiACT Q-6, specific surface area: 505 m²/g, pore volume: 0.73 ml/g, particle size: 75-150 μm and pore size: 6 nm), which is a spherical mesoporous material with a pore diameter of 6 nm, was a gift from Fuji Silysia Chemical, Ltd., Japan, and used as received.

2.2. Preparation of the fucoxanthin-silica gel conjugate (Fx-CARiACT)

0.4 – 0.8 mL of the benzene solution of fucoxanthin (0 – 1.6 mM) was dropped on 10-20 mg of CARiACT in glass tube. Fucoxanthin was adsorbed to the pores of CARiACT following sonication for about 1 minute. After the fucoxanthin-silica gel

conjugate (Fx-CARiACT) precipitated, supernatant benzene solution was removed and kept. The Fx-CARiACT was washed by adding pure benzene solution (1 – 2 mL) to it, and their supernatant was again removed and saved. This washing process was repeated 3-4 times. Then, Fx-CARiACT was dried under reduced pressure for an hour. The amount of fucoxanthin adsorbed to the pores of CARiACT was determined by measuring the absorbance of pooled supernatant benzene solutions at 460 nm, which is the absorption maximum of fucoxanthin in benzene. The molar extinction coefficient of fucoxanthin was reported to be $1.09 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ at 443 nm in acetone [25].

2.3. Optical characterization

All optical measurements were performed at room temperature and in the air. Fx-CARiACT samples were dispersed in a 2 M sucrose solution and then subjected to spectroscopic measurements. Electronic absorption spectra were recorded using a JASCO V670 spectrophotometer, and circular dichroism (CD) spectra were recorded using a JASCO J-720W spectropolarimeter. The sucrose solutions of CARiACT were transparent. However, in order to avoid unnecessary precipitation of Fx-CARiACT in sucrose solution, spectral measurements were performed with a high scanning speed; 1200 nm/min for the absorption and 500 nm/min for the CD measurements.

3. Theoretical Calculations

The magnitude of the transition dipole moment of fucoxanthin, which is necessary to determine the molecular arrangement in its aggregate, was determined by the following calculations. The oscillator strength f of the absorption transition of fucoxanthin was obtained by the following equation [26].

$$f = 4.32 \times 10^{-9} n_r \int_{\nu_1}^{\nu_2} \epsilon(\nu) d\nu \quad (1),$$

where n_r is the refractive index of the solvent and $\epsilon(\nu)$ is the molar extinction coefficient as a function of wavenumber ν . The relation between the oscillator strength and the transition dipole moment is expressed as follows [27].

$$f = \frac{8\pi^2 m_e c v_{nm}}{3 h e^2} |\mu|^2 \quad (2),$$

where m_e is the mass of electron, e is the electric charge of electron, h is Planck's constant, c is the speed of light, v_{nm} is the energy of the particular ($n \leftarrow m$) transition in cm^{-1} , and μ is the transition dipole moment in Debyes. Therefore, The magnitude of the transition dipole moment can be rewritten as:

$$|\mu| = 1458.4 \sqrt{\frac{f}{v_{nm}}} \quad (3).$$

In order to discuss the molecular arrangement of a fucoxanthin dimer, which is considered to form an aggregate, we employed the following approximation based on the excitonic model [6, 7, 10]. According to the point-dipole approximation, the interaction energy (ΔE) between the neighboring transition dipole moments mainly depends on the magnitude of their transition dipole moment, intermolecular distance and molecular orientation, which is expressed as follows.

$$\Delta E \approx \frac{2(N-1)}{N} 5.03 \times 10^3 \left(\frac{f_n^2}{n^2} \right) \frac{|\mu|^2}{|\mathbf{R}_{21}|^3} (\cos\theta - 3\cos\alpha \cos\beta) \quad [\text{cm}^{-1}] \quad (4),$$

where N is the aggregation number, $\mathbf{R}_{21}$ is the distance between two transition dipole moment vectors in Å, θ is the angle between these vectors, and α and β are the angles between each of transition dipoles and $\mathbf{R}_{21}$ (see Figure 1). f_n is a local field correction factor that is expressed as $f_n = (n^2 + 2)/3$, where n is the refractive index of the medium.

4. Results and discussion

4.1. Adsorption efficiency of fucoxanthin to CARiACT

Figure 2 shows a double logarithmic plot that shows the relation between the amounts of fucoxanthin added to and adsorbed on CARiACT. Table 1 summarizes the amounts of addition, adsorption, and adsorption efficiency (%). The amount of adsorbed fucoxanthin to CARiACT increases proportionally to the amount of fucoxanthin added in the range shown in Figure 2(a)-2(d). However, the amount of adsorption deviates in the case of Figure 2(e). The efficiency of adsorption was 100 % in the cases of Figure 2(a)-2(d),

however, it decreased to 61.1% in the case of Figure 2(e) (see Table 1). In this case of Figure 2(e) the exact amount of adsorbed fucoxanthin is calculated to be 7.37×10^{-5} mol/g. Since the pore volume of CARiACT Q-6 is 0.73 mL/g, the density of fucoxanthin in the pores is calculated to be 1.01×10^{-4} mol/mL. When the fucoxanthin molecules are assumed to be uniformly distributed in the pores, the averaged intermolecular distance between fucoxanthin molecules in the pore can be estimated to be 2.54 nm. Therefore, in the case of Fx-CARiACT (e) (Figure 2(e)) the pores of CARiACT are probably filled and saturated with fucoxanthin.

4.2. UV/Vis spectra of Fx-CARiACT

Figure 3 shows the normalized UV/Vis absorption spectra of fucoxanthin in Fx-CARiACT (a)-(e). Absorption spectrum of fucoxanthin in benzene solution is also displayed for comparison. The absorption spectrum of fucoxanthin in benzene corresponds to that of monomer form. The refractive index of benzene (1.50) is similar to that of silicon dioxide (~ 1.5). When the amounts of adsorbed fucoxanthin were relatively small, the main absorption bands of fucoxanthin in Fx-CARiACT show a blue shift compared with that in benzene solution (see Figure 3(a) and 3(b)). This is a good indication that fucoxanthin forms H-aggregates (card-pack type aggregates) in Fx-CARiACT (a) and (b) [28]. On the other hand, when the amount of adsorption was increased, the absorption intensity at ~ 415 nm decreased and a large red-shift of absorption bands occurs (See Figure 3(c), 3(d) and 3(e)). This suggests the formation of J-aggregates (head-to-tail type aggregates) of fucoxanthin in Fx-CARiACT (c), (d), and (e) [28]. Further, the main absorption bands were shifted toward longer wavelengths as the amount of adsorbed fucoxanthin increased. In other words, the main absorption bands of the J-aggregates shifted to longer wavelengths as the molecular density of fucoxanthin in the pores of CARiACT increased. The absorption maximum of Fx-CARiACT (c), (d) and (e) were 532, 545 and 570 nm, respectively; these values were precisely determined by taking the second derivative waveforms of the absorption spectra. These large red-shifts of the absorption maximum are comparable to that of fucoxanthin in FCP [4, 5, 29], a fact which may show that the large red-shift of absorption of fucoxanthin in FCP could be caused by similar intermolecular interactions of fucoxanthin molecules. This idea is supported by the fact 5 molecules of fucoxanthin are bound to single FCP [30].

4.3. CD spectra of Fx-CARiACT

Figure 4 shows the UV/Vis and CD spectra of Fx-CARiACT (b) in which fucoxanthin molecules form H-aggregate. The CD spectrum shows a *left-handed* chiral exciton couplet corresponding to the blue-shifted absorption band of Fx-CARiACT (b) at 415 nm. This is well known as the typical exciton couplet observed for H-aggregates of carotenoids [9-11,13,15]. Such an exciton couplet, classified into negative chirality, means that mutual direction of the transition dipole moments of fucoxanthin in the aggregate twist in a counterclockwise direction. The fucoxanthin molecules in the H-aggregate are form the molecular arrays with a *left-handed* helical structure. On the other hand, Fx-CARiACT (c)-(e) did not show such a bisignate couplet in their CD spectra (data not shown).

4.4. Molecular arrangement of fucoxanthin in the H aggregate as estimated using the point-dipole approximation

To determine intermolecular distance and molecular orientation of fucoxanthin molecules in the H-aggregate in the pores of CARiACT, we performed the following calculations [10, 26]. Figure 5 shows the absorption spectrum of fucoxanthin dissolved in benzene. First, we performed fitting of this absorption spectrum using five Voigt functions in order to numerically calculate the magnitude of the $S_0 \rightarrow S_2$ transition dipole moment of fucoxanthin. The parameters for the five Voigt functions are summarized in Table 2. As a result using Eq. (1) and (2), the magnitude ($|\mu|$) of transition dipole moment of fucoxanthin was determined to be 18.4 Debyes. Further, since the locations of the 0-0 absorption band of fucoxanthin in benzene and the absorption band of H-aggregated fucoxanthin were 20367 cm^{-1} (491 nm) and 24096 cm^{-1} (415 nm), respectively, the interaction energy (ΔE) between fucoxanthin molecules forming the H-aggregate was calculated to be 3729 cm^{-1} .

Then, we adopted the following approximation, which has been proposed by Zsila *et al.* for the H-aggregates of lutein in aqueous acetone [10]. First, if we assume $\theta = 0^\circ$ and $\alpha = \beta = 90^\circ$ in Figure 1(b) (card-packed type arrangement), the intermolecular distance of the fucoxanthins in the dimer can be estimated to be $7.4 \text{ \AA}$, according to Eq. (4) (N in Eq.

(4) is assumed to be 2). Here, we used a refractive index of $n = 1.5$. This value of $7.4 \text{ \AA}$ corresponds to the distance between the planes of the conjugated C=C double bonds of fucoxanthin. However, we must take the spatial extension of the end groups attached to the conjugated C=C double bonds of fucoxanthin into consideration. Figure 6 shows the size of fucoxanthin molecule represented by the van der Waals radius. If two fucoxanthin molecules approach to the distance of $7.4 \text{ \AA}$ while maintaining $\theta = 0^\circ$ and $\alpha = \beta = 90^\circ$, severe spatial hindrance of end groups of each of two fucoxanthin molecules would take place. Therefore, the polyene chains of fucoxanthin molecules should be twisted ($\theta \neq 0^\circ$) so as not cause steric hindrance. Moreover, the bisignate Cotton effect in CD spectrum of the H-aggregate suggests that the dimer of fucoxanthin has a *left-handed* helical structure. Taking this into consideration and when θ is as large as 35° , the overlap of the end groups would be fully avoided. This is obviously the upper limit of the twisting, and the calculated intermolecular distance when $\theta = 35^\circ$ and $\alpha = \beta = 90^\circ$ becomes $6.9 \text{ \AA}$. This separation is sufficient to accommodate two fucoxanthin molecules in card-packed manner by taking the thickness of the conjugated double bond chain ($2.8 \text{ \AA}$ as illustrated in Figure 1(b)) into consideration. Based on the above considerations, it can be safely concluded that the fucoxanthin molecules in the H-aggregates should be twisted by up to 35° and separated by about $6.9 \text{ \AA}$ with respect to each other.

5. Conclusion

In the present investigation, we were successful to prepare fucoxanthin-silica gel (CARiACT) conjugates. When the amount of adsorbed fucoxanthin to the pores of CARiACT was relatively small, fucoxanthin molecules showed the formation of H-aggregates. In contrast, when the amount of adsorption increased, the adsorbed fucoxanthin showed a trend to form J-aggregates. The major absorption bands of J-aggregates showed the large red shifts when the molecular density of fucoxanthin in the pores of CARiACT was increased, *i.e.* the intermolecular distances between fucoxanthin molecules became short. Therefore the large shift of fucoxanthin in FCP can be explained by the dipole-dipole interaction of neighboring fucoxanthin molecules.

In the CD spectrum of Fx-CARiACT, which mainly encapsulates H-aggregates of fucoxanthin, we observed a *left-handed* chiral exciton couplet corresponding to the

blue-shifted absorption band of Fx-CARiACT. This is a common feature typical of H-aggregates of carotenoids. On the other hand, in the case of the J-aggregates, this signature was not observed. We have, therefore, demonstrated how to control which of two types of aggregates form in Fx-CARiACT.

Further, we have been able to calculate the molecular arrangement of fucoxanthin molecules in the H-aggregate based on a point-dipole model. The fucoxanthin molecules in H-aggregates have a *left-handed* helical structure maintaining an intermolecular distance as large as 6.9 Å and the torsion angle smaller than 35°.

Thus, we could observe and control of the aggregates of fucoxanthin, which have never been reported for self-association case in solution phase. The conjugate of carotenoids and mesoporous materials is a promising tool with which to control the molecular arrangement and hence the intermolecular interactions of carotenoids in aggregates.

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Figure Captions

Figure 1. (a) Chemical structure of fucoxanthin and (b) a model that shows the arrangement of fucoxanthin in a dimer.

Figure 2. A double logarithmic plot that shows the relation between the amounts of fucoxanthin added to and adsorbed on CARiACT. The horizontal axis shows the amounts of fucoxanthin added to 1 mg of CARiACT, while the vertical axis shows the amounts of fucoxanthin adsorbed on 1 mg of CARiACT. The label (a), (b), (c), (d), and (e), respectively, correspond to Fx-CARiACT (a), (b), (c), (d), and (e) in Table 1. Broken line is the linear extrapolation of the points that show the 100% adsorption rate.

Figure 3. Normalized UV/Vis absorption spectra of fucoxanthin in Fx-CARiACT (a)-(e) in 2 M sucrose solution. Broken line shows the absorption spectrum of fucoxanthin in benzene solution.

Figure 4. UV/Vis absorption (upper panel) and circular dichroism (CD, lower panel) spectra of fucoxanthin in Fx-CARiACT (b) in 2 M sucrose solution. The CD spectrum was obtained by the average of 16 independent measurements.

Figure 5. Spectral deconvolution of the absorption spectrum of fucoxanthin in benzene using five Voigt functions. The parameters used to create Voigt functions (V1, V2, V3, V4, and V5) are summarized in Table 2. Solid red line shows the experimentally observed absorption spectrum. Blue broken lines show the results of the spectral deconvolution. The sum of the five Voigt profiles is shown with black solid squares.

Figure 6. Connolly surface of a fucoxanthin molecule illustrated with Chem3D software. The lengths in the figure show the thicknesses of either end groups as well as the polyene plane. The length of long axis of fucoxanthin molecule (35 Å) is also shown for convenience. The geometry optimization of fucoxanthin was performed using the MM2 program in Chem3D.

Table 1. Amounts of added and adsorbed fucoxanthin to 1 mg of CARiACT and adsorption efficiency in each case.

	Fucoxanthin added (mmol/mg of CARiACT)	Fucoxanthin adsorbed (mmol/ mg of CARiACT)	Adsorption efficiency (%)
Fx-CARiACT (a)	1.25×10^{-7}	1.25×10^{-7}	100
Fx-CARiACT (b)	1.25×10^{-6}	1.25×10^{-6}	100
Fx-CARiACT (c)	1.21×10^{-5}	1.21×10^{-5}	100
Fx-CARiACT (d)	2.52×10^{-5}	2.52×10^{-5}	100
Fx-CARiACT (e)	1.21×10^{-4}	7.37×10^{-5}	61.1

Table 2. The parameters for five Voigt functions (V1, V2, V3, V4, and V5) that reproduce the shape of absorption spectrum of fucoxanthin in benzene shown in Figure 5.

	V1	V2	V3	V4	V5
Location / cm^{-1}	20367	21692	23041	24450	25913
Amplitude	0.620	0.821	0.638	0.364	0.199
FWHM / cm^{-1}	1385	1528	1664	1658	1608

Figure 1. (a) Chemical structure of fucoxanthin and (b) a model that shows the arrangement of fucoxanthin in a dimer.

Figure 2. A double logarithmic plot that shows the relation between the amounts of fucoxanthin added to and adsorbed on CARiACT. The horizontal axis shows the amounts of fucoxanthin added to 1 mg of CARiACT, while the vertical axis shows the amounts of fucoxanthin adsorbed on 1 mg of CARiACT. The label (a), (b), (c), (d), and (e), respectively, correspond to Fx-CARiACT (a), (b), (c), (d), and (e) in Table 1. Broken line is the linear extrapolation of the points that show the 100% adsorption rate.

Figure 3. Normalized UV/Vis absorption spectra of fucoxanthin in Fx-CARiACT (a)-(e) in 2 M sucrose solution. Broken line shows the absorption spectrum of fucoxanthin in benzene solution.

Figure 4. UV/Vis absorption (upper panel) and circular dichroism (CD, lower panel) spectra of fucoxanthin in Fx-CARiACT (b) in 2 M sucrose solution. The CD spectrum was obtained by the average of 16 independent measurements.

Figure 5. Spectral deconvolution of the absorption spectrum of fucoxanthin in benzene using five Voigt functions. The parameters used to create Voigt functions (V1, V2, V3, V4, and V5) are summarized in Table 2. Solid red line shows the experimentally observed absorption spectrum. Blue broken lines show the results of the spectral deconvolution. The sum of the five Voigt profiles is shown with black solid squares.

Figure 6. Connolly surface of a fucoxanthin molecule illustrated with Chem3D software. The lengths in the figure show the thicknesses of either end groups as well as the polyene plane. The length of long axis of fucoxanthin molecule (35 Å) is also shown for convenience. The geometry optimization of fucoxanthin was performed using the MM2 program in Chem3D.

Graphical Abstract