Spectral tuning and excitation-energy transfer by unique carotenoids in diatom light-harvesting antenna

Kazuhiro J. Fujimoto,1,2* Takuya Seki,2 Takumi Minoda,2 and Takeshi Yanai1,2*

1 Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Furocho, Chikusa, Nagoya 464-8601, Japan. 2 Department of Chemistry, Graduate School of Science, Nagoya University, Furocho, Chikusa, Nagoya, 464-8601, Japan.

ABSTRACT: The light-harvesting antennae of diatoms and spinach are composed of similar chromophores; however, they exhibit different absorption wavelengths. Recent advances in cryoelectron microscopy have revealed that the diatom light-harvesting antenna fucoxanthin chlorophyll a/c-binding protein (FCPII) forms a tetramer and differs from the spinach antenna in terms of the number of protomers; however, the detailed molecular mechanism remains elusive. Herein, we report the physicochemical factors contributing to the difference in light absorption between the light-harvesting antennae of diatoms and spinach based on spectral calculations using an exciton model. Spectral analysis reveals the significant contribution of unique fucoxanthins (fucoxanthin-S) in FCPII to the diatom-specific spectrum, and further analysis determines their essential role in the excitation-energy transfer to chlorophyll. The findings of this study demonstrate that diatoms employ fucoxanthin-S to harvest energy under the ocean in the absence of long-wavelength sunlight and can provide significant information on the survival strategies of photosynthetic organisms to adjust to their living environment.

INTRODUCTION

Solar energy is converted into chemical energy through photosynthesis, thereby producing organic compounds that are essential for biological activities.1 This process is utilized by various organisms, including cyanobacteria, algae, and plants.2 Photosystem I (PSI) and photosystem II (PSII) protein complexes embedded in the thylakoid membrane perform the central reactions of photosynthesis and undergo charge-separation reactions at their reaction centers.1,2 Photosynthetic organisms utilize light-harvesting antenna proteins surrounding the PSII cores to efficiently gather the required light energy for these reactions. The additional use of a light-harvesting antenna enables light absorption over a wider wavelength range than that of the PSII core alone, resulting in a more efficient and stable energy supply to the reaction center. Although numerous PSII core components are conserved from cyanobacteria to plants, the components of the light-harvesting antennae vary widely among photosynthetic organisms.3-5 This implies that photosynthetic organisms flexibly adjust the absorption wavelength of their light-harvesting antennae in response to their environment.

During the evolution of oxygenic photosynthesis, light-harvesting antennae primarily developed into red and green lineages6 by modifying their absorption wavelengths. The types of light-harvesting antennae are associated with the body color of photosynthetic organisms. Diatoms and brown algae belong to the red lineage, whereas green algae and plants belong to the green lineage. The light-harvesting antennae of diatoms and spinach exhibit different absorbances in the wavelength range of 500–560 nm, which is considered to be responsible for the differences in body color between them.7-8 Notably, these spectral differences in light-harvesting antennae are produced by chromophores with structurally similar chemical compositions. For instance, the light-harvesting antennae of diatoms contain fucoxanthin and chlorophyll a as carotenoids and chlorophyll pigments, respectively, which are analogous in overall skeletal structure to neoxanthin and chlorophyll b, respectively, in the spinach antennae (Figure 1A). These observations imply that the spectral differences between diatoms and spinach are due to the slight structural differences in the chromophores or differences in the chromophore-protein interactions. However, the detailed mechanisms causing these differences remain unclear.

Recent advances in cryoelectron microscopy (cryo-EM)9 have contributed to the three-dimensional structural determination of protein complexes in various types of light-harvesting antennae.10-14 These findings enable theoretical and computational investigations of the spectral tuning mechanisms of light-harvesting antennae. However, the large size of these protein systems renders general quantum chemical calculations impractical.

The exciton model,15 which calculates the electronic state of the entire system from the electronic states of the individual molecular fragments, is an effective approach for solving this problem in terms of computational cost and accuracy. Previous studies applied the exciton model to tetracene crystals,16 retinal proteins,17 and the light-harvesting 2 complex (LH2)18 and obtained reliable predictions of their optical properties, thereby demonstrating its effectiveness on large-scale systems.

https://doi.org/10.26434/chemrxiv-2023-52xpr ORCID: https://orcid.org/0000-0003-0286-3646 Content not peer-reviewed by ChemRxiv. License: CC BY-NC-ND 4.0
This study aimed to elucidate the molecular mechanisms of spectral tuning in the light-harvesting antennae of diatoms (Chaetoceros gracilis) and spinach (Spinacia oleracea), which are representative of the red and green lineages, respectively. The light-harvesting antenna of diatoms is called fucoxanthin chlorophyll $a/c$-binding protein (FCPII) and is composed of fucoxanthin, chlorophyll $a$, and chlorophyll $c$ (Figure S1). By contrast, spinach contains a light-harvesting pigment-protein complex (LHCII) consisting of lutein, neoxanthin, violaxanthin, chlorophyll $a$, and chlorophyll $b$ (Figure S1). Nagao et al. discovered that FCPII forms a tetramer, which differs from LHCII (a trimer) in the number of constituent protomers. This difference in the aggregate structure may be responsible for the distinct spectral tuning of the two light-harvesting antennae. To investigate the molecular mechanisms of the two light-harvesting antennae, first, this study attempted to reproduce the experimental absorption spectra of the diatom FCPII tetramer and the spinach LHCII trimer using the exciton theory with the first-principles computational modeling. Next, the simulated spectra were compared in detail to determine the physicochemical factors underlying the distinct spectral tuning of the two light-harvesting antennae. Furthermore, the electronic couplings between the chromophores were analyzed to determine their role in realizing efficient photosynthetic excitation-energy transfer (EET) in the light-harvesting antenna. Based on the resulting molecular-level descriptions, deeper biological insights into the survival strategies of photosynthetic organisms were derived.

**RESULTS AND DISCUSSION**

**Absorption Spectra Calculated with the Exciton Model.** The experimental structures were refined using molecular dynamics (MD) simulations, followed by geometry optimization using the ONIOM (short for "our own n-layered integrated molecular orbital and molecular mechanics") method, presented in detail in the Supporting Information. The optimized structures of the FCPII tetramer and the LHCII trimer are shown in Figure 1B. Compared with the experimental structures, these refinements did not alter the overall structure of the system significantly. However, the carotenoid $\pi$-chain exhibited a certain degree of structural differences, such as a slightly reduced bond length alternation (BLA) in the optimized structure and a stronger BLA in the experimental structure (Figure S2).
The absorption spectra of the optimized structures were simulated using the exciton model; details of the exciton model calculations are provided in the Supporting Information. Figure 2A depicts the calculated absorption spectra of the FCPII tetramer and the LHCII trimer. The FCPII tetramer exhibited a small peak at 620 nm, large peaks at 450–500 nm, and an intermediate peak at 527 nm. By contrast, the LHCII trimer exhibited small peaks at 615 and 516 nm, and a large peak at 450–500 nm. These calculations afforded reproduced shapes of the experimental spectra that were not identical but were satisfactory (Figure 2B). Using the absorption peak at 620 nm as a reference, the calculated absorption intensity of the FCPII tetramer at 500–560 nm was greater than that of the LHCII.

**Figure 2.** Absorption spectra. (A) Absorption spectra of the FCPII tetramer and LHCII trimer calculated using the exciton model. (B) Experimental absorption spectra of FCPII and LHCII taken from Fig. 2 of Ref. 1. (C) Convolution spectra calculated by summing the contributions of each protomer and absorption spectrum calculated by removing the electrostatic (ES) interactions between protomers from the FCPII tetramer. (D) Absorption spectra of each chromophore in the FCPII tetramer calculated using the exciton model. (E) Absorption spectra calculated by removing the contribution of fucoxanthin-S from the FCPII tetramer and by replacing fucoxanthin-S with neoxanthin in the FCPII tetramer. The 500–560 nm region is highlighted in light green.
trimer. These results suggest that the calculated spectra are reliable for providing details on the factors that cause the differences between the spectra of diatoms and spinach.

As mentioned earlier, the primary structural difference between FCPII and LHClI is the multimeric form; FCPII and LHClI form a tetramer and trimer, respectively. The aforementioned spectra of all complexes are denoted herein as multimeric spectra (Figure 2A). Next, the convolution spectra were obtained via a simple sum of all the absorption spectra of the monomeric protomers calculated using the exciton model (Figure 2C). Thus, in the convolution spectra, the multimeric interactions between the protomers were discarded. The effect of multimerization (or the interaction between protomers) on spectral tuning was examined by comparing the multimeric and convolution spectra (Figure 2C). The comparison revealed a common feature in tetrameric FCPII and trimeric LHClI: although the shapes of the absorption peaks at 450–500 nm differed significantly, the absorption peaks at 620 nm had almost the same shape. These results indicate that the effect of multimerization was greater in the absorption spectrum at 450–500 nm than in the spectrum at 620 nm. Further comparison of the spectra revealed a new absorption peak at 500–560 nm upon FCPII tetramerization, whereas only a slight change in the spectral shape was observed upon LHClI trimerization. These results strongly suggest that the absorption at 500–560 nm is associated with the tetramerization of FCPII.

The contribution of each chromophore to the overall spectrum of the FCP tetramer was analyzed. The spectra of each chromophore were calculated using an exciton model that incorporates chromophore interactions between the same species without considering chromophore interactions between other species. The resulting spectra are shown in Figure 2D. Spectral analysis revealed that the absorption peak at 620 nm of the FCPII tetramer was attributed to chlorophyll a, and the peaks at 450–500 nm and 527 nm were generated by fucoxanthin. These findings strongly suggest that fucoxanthin is responsible for the absorbance at 500–560 nm of the FCPII tetramer, which is associated with the reddish-brown color of diatoms.

To further examine the characteristics of fucoxanthin, its location in FCPII was investigated. As illustrated in Figure 1C, the protomeric structures of FCPII and LHClI were similar, constituting three helices (α1, α2, and α3).19 Comparisons of the carotenoid positions in both proteins revealed the presence of a specific fucoxanthin between α1 and α2 in FCPII, referred to as fucoxanthin-S, whereas no such carotenoid was detected in LHClI. Four fucoxanthin-S molecules were identified in the FCPII tetramer.

To investigate the effect of fucoxanthin-S, a numerical experiment was performed by calculating the absorption spectra of a modified FCPII tetramer, from which only four fucoxanthin-S molecules were removed. As shown in Figure 2E, the resulting spectrum exhibited a reduced peak at 500–560 nm compared with the original FCPII tetramer spectrum. These results indicate that fucoxanthin-S plays an essential role in the absorption at 500–560 nm.

The obtained results highlighting the importance of fucoxanthin-S led to the idea of replacing fucoxanthin-S with other carotenoids and the following calculations were therefore conducted: First, a modified FCPII tetramer was prepared, in which the four fucoxanthins-S were replaced with neoxanthins (Figure 1A). ONIOM geometry optimization was then performed for each neoxanthin molecule, and the resulting neoxanthin-substituted FCPII tetramer was used to calculate the absorption spectrum. As shown in Figure 2E, the neoxanthin-substituted FCPII tetramer exhibited an absorption peak at 500–560 nm, similar to that of the original FCPII tetramer. From the above analysis, it was concluded that the position of fucoxanthin-S, rather than the type of carotenoid, was the major contributor to the absorption at 500–560 nm of the FCPII tetramer.

Spectral analysis revealed the significant role of tetramerization and fucoxanthin-S on the absorption at 500–560

**Figure 3.** ES energy analysis. (A) The ES energy contribution of each molecule (amino acid or chromophore) to the spectral shift of fucoxanthin-S in the FCPII tetramer. Molecules with large redshift contributions are shown in red ($E_{A}^{ES} < -0.1$ eV) and orange ($-0.1$ eV < $E_{A}^{ES} < -0.05$ eV), while molecules with large blueshift contributions are shown in blue ($E_{A}^{ES} > 0.1$ eV) and cyan (0.05 eV < $E_{A}^{ES} < 0.1$ eV). (B) Graphical representation of the ES energy contribution ($|E_{A}^{ES}| > 0.05$ eV). The positive and negative values represent the contribution to the blueshift and redshift, respectively.
nm in FCPII. However, the relationship between these factors remains unclear. Additionally, the contribution of fucoxanthin-S could not be clarified because the convolution spectrum did not show absorption at 500–560 nm, despite the incorporation of the contribution of fucoxanthin-S (Figure 2C). As noted before, the convolution spectra were computed by including the intraprotomer interactions, but not the interprotomer interactions. To examine the relationship between tetramerization and fucoxanthin-S, the absorption spectrum was calculated using a modified exciton model, in which only the electrostatic (ES) effects between the FCPII protomers were removed, and the resulting spectra were compared with those of the exciton model. As shown in Figure 2C, the absorption at 500–560 nm disappeared from the spectrum calculated using the modified exciton model, which is significantly different from the results obtained using the exciton model. It was thus concluded that the absorption in the 500–560 nm range in FCPII resulted from the interprotomer ES interaction between fucoxanthin-S and the tetramerized protein, rather than from the intraprotomer ES interaction.

To gain further insight into the interprotomer ES interactions, the ES energy contribution of each molecule (amino acid or chromophore) to the spectral shift was calculated according to Eq. (1).

\[
E_{ES}^{A} = \sum_{i \in A} \sum_{j \in Fuco-S} \frac{Q_{i} (q_{j}^{e} - q_{j}^{g})}{4\pi\varepsilon_{0}r_{ij}}
\]

where, \(Q_{i}\) is the charge of atom \(i\) in molecule \(A\), \(q_{j}^{e}\) and \(q_{j}^{g}\) are the charges of atom \(j\) in the fucoxanthin-S chromophore in the excited and ground states, respectively, \(\varepsilon_{0}\) is the vacuum permittivity, and \(r_{ij}\) is the interatomic distance.

Electronic Couplings of Fucoxanthin-S. Fucoxanthin-S was present in FCPII, whereas no such carotenoid was found at similar positions in LHCII. The electronic coupling of fucoxanthin-S was analyzed to further investigate its role in the light-harvesting antenna FCPII. Electronic coupling \(V\) is an intermediate physical quantity that represents the intermolecular interactions between different electronic states, and the square of the electronic coupling is an essential factor in determining the EET rate constant. In the exciton model for the absorption spectrum calculation, the electronic coupling \(V\) corresponds to the off-diagonal element in the exciton Hamiltonian matrix, described by Eq. (2).

\[
F_{\text{TrESP}}(\text{cm}^{-1}) \quad R_{AB} (\text{Å}) \quad \kappa \quad \theta_{AB} (\text{degrees}) \quad \theta_{A} (\text{degrees}) \quad \theta_{B} (\text{degrees})
\]

| Fucoxanthin-S - Chlorophyll a | 529.8 | 7.1 | 0.448 | 9.1 | 61.0 | 68.2 |
| Normal fucoxanthin - Chlorophyll a/c | ~271.9 | - | - | - | - | - |

**Figure 4.** Analysis of the electronic couplings. (A) Electronic couplings calculated using the TrESP method and the parameters involved in electronic coupling. (B) Definition of three angles \(\theta_{AB}\), \(\theta_{A}\), and \(\theta_{B}\) in the orientation factor \(\kappa\). (C) Transition dipole moments of fucoxanthin-S and chlorophyll a.
Figure 5. Light energy harvesting by fucoxanthin and fucoxanthin-S. (A) Irradiance transmittance at ocean depths of 1–25 m. The diffuse attenuation coefficient for irradiance at each wavelength is taken from Ref. 36. (B) Schematic illustration of the EET path in the FCPII tetramer. EET from normal fucoxanthin to fucoxanthin-S and then to chlorophyll a is proposed from the obtained results.

\[ V = \langle \Phi_i | H | \Phi_j \rangle \]  
\[ \kappa = \cos \theta_{\|} - 3 \cos \theta_A \cos \theta_B \]  

where \( H \) represents the total Hamiltonian of an assembly system; and \( \Phi_i \) and \( \Phi_j \) represent the basis states. Although various methods have been proposed to calculate the electronic couplings, herein, the transition charge from electrostatic potential (TrESP) method was adopted, in which the electronic coupling \( V \) is described by Eq. (3), a typical Coulomb interaction.

\[ V_{\text{TrESP}} = \sum_{i \neq j} \sum_r \frac{q_i q_j}{4 \pi \epsilon_0 r_{ij}^3} \]  

where \( q_i \) is the transition charge of atom \( i \) in molecule A. All the electronic couplings in FCPII involving fucoxanthin-S were calculated using Eq. (3). Fucoxanthin-S exhibited a larger value of electronic coupling with chlorophyll a, with the maximum value calculated to be 529.8 cm\(^{-1}\) (Figure 4A). This value was much larger than the normal fucoxanthin-chlorophyll electronic couplings in FCPII (272.0 cm\(^{-1}\)) and all the carotenoid-chlorophyll electronic couplings in LHCl (Table S1 and S2). These results imply that fucoxanthin-S plays an important role in the EET to chlorophyll a as well as in the absorption spectrum of FCPII.

To further investigate the factors contributing to the large electronic coupling between fucoxanthin-S and chlorophyll a, we analyzed the electronic coupling in terms of the dipole-dipole (DD) approximation. The DD approximation, which is the simplest way to calculate electronic coupling, is described by Eq. (4).

\[ V_{\text{DD}} = \frac{|\mu_A| |\mu_B| \kappa}{4 \pi \epsilon_0 R_{AB}^3} \]  

where \( \mu_A \) is the transition dipole moment of molecule A; \( R_{AB} \) is the distance between molecules A and B; and \( \kappa \) is the orientation factor determined by the angles (\( \theta_A \), \( \theta_B \), and \( \phi_{AB} \)) involved in the transition dipole moments of molecules A and B (Figure 4B). The results obtained by applying Eq. (4) for the fucoxanthin-S and chlorophyll a pair (which exhibited the largest electronic coupling), are summarized in Figure 4A. The intermolecular distance \( R_{AB} \) was calculated to be 7.1 Å and the angle between the two transition dipole moments \( \theta_{AB} \) was calculated to be 9.1 degrees (Figure 4C). These results indicate that the close distance and nearly parallel orientation of fucoxanthin-S and chlorophyll a can be attributed to the large electronic coupling.

Role of Fucoxanthin-S in Realizing Efficient Photosynthetic EET. The absorption spectra of the FCPII tetramer and LHCl trimer were analyzed in the context of their biological significance. Diatoms containing the FCPII tetramer have been found in the sea at a depth of approximately 5 m near the coast, whereas spinach containing the LHCl trimer is a land plant. In oceans deeper than 5 m, the sunlight intensity at wavelengths longer than 600 nm is significantly reduced (Figure 5A). As chlorophyll absorbs light with wavelengths longer than 600 nm, diatoms living at a depth of 5 m cannot obtain the sunlight energy necessary for photosynthesis from chlorophyll alone. To obtain sufficient light energy, diatoms may utilize the carotenoids in FCPII as light absorbers. Previous studies have shown that the EET between the light harvesting antenna and the PS is mediated by chlorophyll a, where light energy is collected in chlorophyll a. As mentioned earlier, the largest electronic coupling of fucoxanthin-S was observed for chlorophyll a (Figure 4A), and the electronic couplings between fucoxanthin-S and normal fucoxanthin...
were also large (Table S3). These results combined with the calculated energy levels of the absorption spectra (Figure 2) suggest that the EET path within the FCPII tetramer is from normal fucoxanthin to fucoxanthin-S and then to chlorophyll a (Figure 5B). These findings suggest that fucoxanthin-S compensates for the inadequate light-harvesting function of chlorophyll in diatoms. Furthermore, fucoxanthin-S is believed to play an essential role as an EET bridge between normal fucoxanthin and chlorophyll a. By contrast, chlorophyll in spinach can directly obtain sufficient light energy for photosynthesis because sunlight with wavelengths longer than 600 nm is not blocked on the ground. Therefore, spinach can maintain its biological activity without utilizing carotenoid light absorption at 500–560 nm.

CONCLUSIONS
This study aimed to elucidate the difference in spectral tuning between the FCPII tetramer and LHCII trimer using the exciton model. The calculated absorption spectra of the FCPII tetramer and the LHCII trimer reproduced the shapes of the experimental spectra, corroborating the difference in absorption intensity at 500–560 nm. Moreover, spectral analyses revealed that the absorption at 500–560 nm, which is associated with the reddish-brown color of the diatoms, was attributed to fucoxanthin-S in the FCPII tetramer. The obtained data highlight the importance of both FCPII tetramerization and fucoxanthin-S for absorption at 500–560 nm in diatoms. Further analysis of electronic coupling demonstrated that fucoxanthin-S in the FCPII tetramer was more likely to cause EET to chlorophyll a than the carotenoids in the LHCII trimer. Additionally, the present study reveals the important role of fucoxanthin-S as an EET bridge between normal fucoxanthin and chlorophyll a. These data support the hypothesis that the light-harvesting antennae of diatoms utilize the optical properties of carotenoids to obtain sufficient light energy for photosynthesis, even in the absence of long-wavelength sunlight. These findings have significant implications for understanding the survival strategies of photosynthetic organisms while adjusting to their living environments.

ASSOCIATED CONTENT
Supporting Information.
This material is available free of charge via the Internet at http://pubs.acs.org.
Details of the computational procedures; supporting figures (Figure S1–S2); supporting tables (Table S1–S3); and the atomic coordinates of the optimized chromophores (PDF)

AUTHOR INFORMATION
Corresponding Author
* fujimotok@chem.nagoya-u.ac.jp (K.J.F.)
* yanait@chem.nagoya-u.ac.jp (Y.T.)

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENT
This study was supported by JSPS KAKENHI (Grant No. 20K05430 to K.J.F. and 21H01881 and JP21K18931 to T.Y.), JST, CREST (Grant No. JPMJCR2105 to K.J.F.).

REFERENCES


