Conformational Changes in Light-Harvesting Complex II Trimer Depending on Its Binding Location

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Abstract

In green plants, the light-harvesting complex II (LHCII) trimer functions as a major antenna complex to the photosystem II (PSII) complex and a quencher to protect it from photooxidative damage. Theoretical studies on the structure of the LHCII trimer have demonstrated that excitation energy transfer between chlorophylls (Chls) can be modulated by its exquisite conformational fluctuation. However, the conformational changes in the LHCII trimer depending on its binding location have not yet been investigated, even though protein-protein interactions tend to lead conformational changes. In this study, we investigated the conformational changes in LHCII by analyzing an identical LHCII trimer comprising three different photosystem supercomplexes—PSII–LHCII supercomplexes (C₂S₂ type and C₂S₂M₂L₂ type) and PSI–LHCI–LHCII supercomplex—from the green alga *Chlamydomonas reinhardtii*. Consequently, the distinct differences in Chl configurations as well as polypeptide conformations of the LHCII trimers were detected based on where LHCII binds. Our analysis of the configurational factors between Chls suggests that these configurational changes lead to higher light-harvesting compatibilities in the PSII–LHCII supercomplex (C₂S₂M₂L₂ type) and PSI–LHCI–LHCII supercomplex. This study suggests a fine-tuned mechanism of energy transfer dynamics in LHCII trimers by regulation of protein-protein interactions.

Introduction

In green plants, chlorophyll (Chl) is the most abundant pigment that is crucially involved in photosynthesis. The first step of photosynthesis is the capture of light by Chls within the pigment-binding proteins embedded in the thylakoid membranes of chloroplasts. The absorbed energy is subsequently transferred to the Chls in the reaction centers of either photosystem I or II (PSI and PSII), thereby energizing the electron transport chain from water to the Calvin-Benson cycle. The most dominant antenna protein in green plants is the light-harvesting complex II (LHCII) that contains 8 Chl a, 6 Chl b, 2 luteins, 1 violaxanthin, and 1 neoxanthin, which can efficiently harvest light in environmental light conditions. Monomeric forms of LHCII only bind to PSII, whereas its trimeric forms (LHCII trimer) bind to PSII and conditionally to PSI to balance the excitation between PSI and PSII.

Structures of the LHCII trimers from spinach have been resolved by crystallography. These molecular structures have provided the Chl configurations that are crucial for theoretical studies of energy transfer dynamics in LHCII trimers. Theoretical analysis combined with spectroscopic analysis revealed that the Chl a610–a611–a612 cluster located on the outer side of the LHCII trimer exhibited predominant population of excitation energy. Therefore, this Chl cluster has been proposed to support efficient energy transfer to the other subunits of PSII. This Chl cluster is also crucially involved in photoprotective energy dissipation. Moreover, Chl a602 and a603 within the stromal-side layer and Chl a613 within the luminal-side layer of the LHCII trimer are crucial in mediating inter-monomeric energy transfers in the LHCII trimer. Furthermore, marvelous features of the LHCII trimer Chls in their electronic states have been revealed. Quantum electronic coherence in the LHCII trimer has been demonstrated by two-dimensional electronic spectroscopy with theoretical studies based on their molecular structures. Moreover, the importance of electronic-vibrational mixing in energy transfer dynamics has been investigated by two-dimensional electronic vibrational spectroscopy. In contrast, multiscale quantum mechanics/molecular mechanics (QM/MM) molecular dynamics simulations have demonstrated that the excitation energy transfer dynamics in LHCII can be modulated by its conformational fluctuation and surrounding environments including pH conditions. These previous reports revealed that the configuration of Chls in the LHCII trimer determines their energy transfer dynamics, and therefore the eventual light-harvesting properties.

Recently, owing to the great advances in cryoelectron microscopy (cryo-EM), PSII–LHCII supercomplexes comprising the PSII core dimer and LHCII complexes (PSII–LHCII), and the PSII–LHCI–LHCII supercomplex comprising the PSI core, LHCl, and LHCII complexes (PSI–LHCI–LHCII) from green plants were resolved. These structures indicated that Chls in LHCII trimers are well connected to the Chls in the core complexes to ensure an efficient light-harvesting process. However, the conformation and Chl configurations of the same LHCII trimers in different supercomplexes have not yet been compared, even though protein-protein interactions can easily transform their protein and ligand structures.

In this study, we investigated the Chl configurations in a LHCII trimer when bound to different supercomplexes. We compared the conformations of an LHCII trimer comprising identical polypeptides, namely, LhcbM1, LhcbM2, and LhcbM3 subunits with two PSII–LHCII supercomplexes—C₂S₂ and C₂S₂M₂L₂—and a PSI–LHCI–LHCII supercomplex from a green alga *Chlamydomonas reinhardtii* (*C. reinhardtii*) (Figure 1A) (Table S1). Moreover, to quantify the difference in the Chl configurations, computational analysis was...
applied to determine the distances and orientation factors between all Chl based on estimated transient dipoles. The effects of the configurational change on site energy and energy transfer dynamics were estimated by calculating energy transfer rate constants based on the Förster resonance energy transfer principle. Based on these results, we propose the way how energy transfer dynamics on LHCII can be modulated by its binding partners.

Results

Conformations of LHCII trimers in three different photosystem supercomplexes

In order to investigate whether the structure of the same LHCII trimer can be affected by where it binds, the polypeptide structures and Chl coordinates of an LHCII trimer comprising LhcbM1, LhcbM2, and LhcbM3 were compared with the two PSII–LHCII supercomplexes—C2S2 and C2S2M2L2—and the PSI–LHCII–LHCII supercomplex from a green alga C. reinhardtii (Figure 1A). The S-trimer in the C2S2 supercomplex (pdb.6KAC) is a hetero trimer of LHCII comprising LhcbM1, LhcbM2, and LhcbM320. Although the polypeptide identities have not been reported in the C2S2M2L2 supercomplex (pdb.6KAD) because of low resolution around the S-trimer region, we assume its S-trimer is also comprises LhcbM1, LhcbM2, and LhcbM3, as in the C2S2 supercomplex. The LHCII-1 trimer in the PSI–LHCII–LHCII supercomplex (pdb.7DZ7) has been reported to be the same hetero trimer, namely, LhcbM1/LhcbM2/LhcbM323. In all three locations, the LhcbM1/LhcbM2/LhcbM3 trimer binds to a core complex with LhcbM1 as the docking site (Figure 1A). The superposed polypeptide structures revealed that the polypeptide structure of the LHCII trimer was not identical when bound to the different supercomplexes (Figure 1B). The S-trimer bound to PSII C2S2M2L2 is slightly stretched to the outside, while the central part stays the same. Similarly, Chls in the S-trimer/C2S2M2L2 are stretched out unlike those in the S-trimer/C2S2 or LHCII-1/PSI–LHCII–LHCII supercomplexes (Figure 1C). This clearly suggests that both the polypeptide structure and the Chl coordinates in an LHCII trimer can vary depending on where it binds.

In order to investigate the flexible domain of the LHCII trimer affected by vicinity, we analyzed the root mean square deviation (RMSD) of the polypeptides between each photosystem supercomplex (Figure 2). Our analysis revealed that the polypeptide conformation of the S-trimer/C2S2M2L2 is considerably different compared to the S-trimer/C2S2 and LHCII-1/PSI–LHCII–LHCII supercomplexes, whereas there was no significant difference between S-trimer/C2S2 and LHCII-1/PSI–LHCII–LHCII supercomplexes (Figure 2A). In the RMSD map between S-trimer/C2S2 and S-trimer/C2S2M2L2, the lumen side of helix B of all LhcbM and helix C/LhcbM2 exhibited high RMSD values. In the RMSD map between S-trimer/C2S2M2L2 and LHCII-1/PSI–LHCII–LHCII, helix C of all LhcbM, helix A of LhcbM2 and LhcbM3, and helix B exposed to the lumen sides of LhcbM2 and LhcbM3 revealed high overall RMSD values. In contrast, the RMSD map between S-trimer/C2S2 and LHCII-1/PSI–LHCII–LHCII revealed lower RMSD values except for the stroma sides of helix A of LhcbM2 and LhcbM3. Figure 2B displays the placements of Chls and carotenoids on the RMSD map between the S-trimer/C2S2M2L2 and LHCII-1/PSI–LHCII–LHCII.

Chls configurations in LHCII trimers of three different photosystem supercomplexes

In order to examine the positional effects on Chl configurations, we superimposed the PDB coordinates and the corresponding electron density maps of a low-energy Chl a611-a612 pair located between helix A and neighbor helix C, in each LHCII subunit of the three different photosystem supercomplexes and displayed their corresponding cryo-EM maps (Figure 3). In LhcbM1, as the RMSD values were found to be low, the relative configuration of the Chl pair and its cryo-EM maps were also found to be analogous. In contrast, in LhcbM2 and LhcbM3, the RMSD values of helix A and helix C were found to be high; therefore, the relative configurations of the Chl pair and the cryo-EM maps differed between photosystem supercomplexes.

Configurational factors such as distance and orientation factors between transient dipole moments of Chls are critical factors for excitation energy transfer dynamics. A weaker dipole interaction induced by a long distance or low orientation factor can result in a lower energy transfer rate. Therefore, we conducted computational analysis for distances and orientation factors between all transient dipole moments of Chls to quantify the effects of different Chl coordinates on Chl configuration. The distance between the central magnesium atoms of the porphyrin rings in Chls was used to calculate the distances and the three-dimensional angles between the unit vectors that were generated based on the coordinates of NB and ND atoms of the porphyrin rings in Chls20,26. Our
comparative analysis clearly indicates that Chl configurations in LHCII trimers are modulated by the polypeptides in the vicinity (Figure 4A), suggesting that the energy transfer properties of the LHCII trimer could be modulated by its binding partner.

In order to estimate the impact of Chl configuration change in the LHCII trimers upon excitation energy transfer dynamics, "interaction factor" was calculated as the energy transfer rate based on the Förster resonance energy transfer (FRET) principle (Figure 4B). In all LHCII subunits, the Chl a611 and a612 pair exhibited the highest interaction factor because of their high orientation factor and relatively short distance (Table 1). Consistent with literature, Chl a611, a612, and a610 were found to be in their lowest energy states because of their strong coupling interactions\(^4,5\). Our results revealed that the interaction factors of the Chl a611-a612 pairs were different in LhcbM2 and LhcbM3, whereas these were similar in LhcbM1 between the three different supercomplexes. In LhcbM2 the interaction factors of the Chl a611-a612 pairs in PSII C2S2M2L2 and PSI–LHCI–LHCII were 21 and 14% lower than that in PSII C2S2, respectively. In LhcbM3, the interaction factors of the Chl a611-a612 pairs in PSII C2S2M2L2 and PSI–LHCI–LHCII were 11 and 13% lower than that in PSII C2S2, respectively. Furthermore, the closest Chl pair—b606-a604—exhibited apparent changes depending on where the LHCII binds. The distance between the Chl b606-a604 pair in LhcbM2 was found to be longer in C2S2M2L2 than that in C2S2 and PSI–LHCI–LHCII.

**Table 1.** Interaction factors between Chl pairs. Significant differences compared to PSII C2S2 are indicated by asterisks. (* > 10% difference, ** > 20% difference, *** > 40%)

| Chl pair | C2S2 | C2S2M2L2 | PSI–LHCI–LHCII |
|----------|------|----------|----------------|
|          | LhcbM1 | LhcbM2 | LhcbM3 | LhcbM1 | LhcbM2 | LhcbM3 | LhcbM1 | LhcbM2 | LhcbM3 |
| Chl a611-a612 | interaction factor | 16.68 | 16.78 | 16.59 | 16.25 | 13.21** | 14.77* | 16.66 | 14.50* | 14.48* |
|            | Distance | 9.57 | 9.50 | 9.57 | 9.64 | 9.60 | 9.84 | 9.45 | 9.66 | 9.66 |
|            | Orientation factor | 2.29 | 2.20 | 2.28 | 2.33 | 1.85* | 2.39 | 2.12 | 2.11 | 2.11 |
| Chl a610-a612 | interaction factor | 0.62 | 0.73 | 0.51 | 0.70* | 0.38*** | 0.55 | 0.64 | 0.34*** | 0.28*** |
|            | Distance | 11.56 | 12.06 | 11.93 | 11.90 | 12.21 | 12.01 | 11.71 | 11.99 | 12.06 |
|            | Orientation factor | 0.26 | 0.40 | 0.26 | 0.35* | 0.22*** | 0.30* | 0.30 | 0.18*** | 0.16** |
| Chl a610-a611 | interaction factor | 0.60 | 0.55 | 0.62 | 0.56 | 0.46* | 0.53* | 0.65 | 0.57 | 0.57 |
|            | Distance | 17.74 | 18.09 | 17.77 | 17.93 | 18.47 | 18.31 | 17.58 | 17.91 | 17.92 |
|            | Orientation factor | 3.34 | 3.48 | 3.51 | 3.34 | 3.25 | 3.54 | 3.42 | 3.37 | 3.38 |
| Chl a613-a614 | interaction factor | 2.91 | 2.92 | 2.65 | 1.62*** | 2.79 | 2.79 | 2.75 | 3.40* | 3.40** |
|            | Distance | 9.32 | 9.35 | 9.45 | 9.60 | 9.56 | 9.61 | 9.47 | 9.64 | 9.64 |
|            | Orientation factor | 0.34 | 0.35 | 0.34 | 0.23** | 0.38 | 0.39** | 0.36 | 0.49*** | 0.49*** |
| Chl a602-a603 | interaction factor | 1.03 | 1.03 | 1.02 | 0.59*** | 0.66** | 0.82** | 0.76** | 0.92* | 0.87* |
|            | Distance | 11.68 | 11.66 | 11.80 | 11.92 | 12.04 | 11.98 | 11.55 | 11.58 | 11.64 |
|            | Orientation factor | 0.47 | 0.46 | 0.49 | 0.30** | 0.36** | 0.44* | 0.32** | 0.40* | 0.39* |
Furthermore, we compared the interaction factors of the other Chl pairs of low energy Chls— the Chl a610-a612 pair and the Chl a610-
a611 pair— (Table 1). Interestingly, the Chl a610-a612 pair was found to be significantly different in LhcbM2 of PSII C_{602}S_{602}M_{602}L_{602} (LhcbM2/C_{602}S_{602}M_{602}L_{602}), LhcbM2/PSI–LHCI–LHCII, and LhcbM3/PSI–LHCI–LHCII. The interaction factors of the Chl a610-a612 pairs in LhcbM2/C_{602}S_{602}M_{602}L_{602} and LhcbM2/PSI–LHCI–LHCII were 48 and 53% lower than that in the LhcbM2/C_{602}S_{602}, respectively, mostly because of lower orientation factors (45 and 55% lower, respectively). However, the interaction factors of Chl a610-a612 pairs in LhcbM3/C_{602}S_{602}M_{602}L_{602} and LhcbM3/PSI–LHCI–LHCII were 8% higher and 45% lower than that in LhcbM3/C_{602}S_{602}. Moreover, the interaction factors of the Chl a610-a611 pairs in LhcbM2 and LhcbM3 of PSII C_{602}S_{602}M_{602}L_{602} were 16 and 15% lower than those in PSI C_{602}S_{602}, respectively, whereas there were no significant differences between PSII C_{602}S_{602} and PSI–LHCI–LHCII.

Although intra-LHCII energy transfer is important for fast energy transfer dynamics, inter-LHCII energy transfer is also crucial as an efficient antenna complex for photosystem core complexes. Here, we investigated the Chl configurations at the interface of the LHCII subunits in the LHCII trimer of the three different photosystem supercomplexes. As shown in the Chl configurations within each LHCII subunit (Figure 3A), Chl configurations between LHCII subunits also varied depending on the vicinity and type of LHCII subunits (Figure 4C). The closest Chls between the LHCII subunits were Chl b601 and b609 in all LhcbM interfaces. However, Chl b rapidly transfers excitation energy to Chl a and the reverse transfer rarely occurs because of the higher energy level of Chl b than that of Chl a; therefore, Chl b601 and b609 are unable to function as major mediators for inter-LHCII energy transfer. We thus focused on the Chl a pairs that are mainly involved in inter-LHCII energy transfer. Consistent with the previous study, our results showed that Chl a602-a603, Chl a603-a613 pairs have higher interaction factors at all LhcbM interfaces (Figure 4D). At the LhcbM1-LhcbM2 interface, the interaction factor of the Chl a602-a603 in PSII C_{602}S_{602}M_{602}L_{602} was found to be higher than those in PSI C_{602}S_{602} and PSI–LHCI–LHCII. In contrast, at the LhcbM1-LhcbM3 interface, the interaction factor of the Chl a602-a603 in PSII C_{602}S_{602}M_{602}L_{602} was found to be lower than those in PSII C_{602}S_{602} and PSI–LHCI–LHCII. This result indicates that energy transfer between LhcbM1 and LhcbM2 is enhanced; however, energy transfer between LhcbM1 and LhcbM3 is suppressed in PSII C_{602}S_{602}M_{602}L_{602} compared to those in PSII C_{602}S_{602} and PSI–LHCI–LHCII. At the LhcbM2-LhcbM3 interface, although the interaction factor of the Chl a602-a603 pair in PSII C_{602}S_{602}M_{602}L_{602} was similar to those in PSI C_{602}S_{602} and PSI–LHCI–LHCII, the interaction factors of the Chl a603-a603 pairs were largely increased in PSII C_{602}S_{602} and PSI–LHCI–LHCII, but not in PSII C_{602}S_{602}M_{602}L_{602}. This result suggests that energy transfer mediated by the Chl a602-a603 pair between LhcbM2 and LhcbM3 is more active in PSI C_{602}S_{602} and PSI–LHCI–LHCII than in PSII C_{602}S_{602}M_{602}L_{602}. Furthermore, we investigated the interaction factors between Chl a603 and other Chls within the LHCII subunit (Table 1), where Chl a603 is supposed to be important in inter-LHCII energy transfer. Consistently, Chl a603 revealed the highest interaction factor with Chl a602 within the overall LHCII subunit. Interestingly, the interaction factors of the Chl a602-a603 pair in PSII C_{602}S_{602} were higher than those in PSII C_{602}S_{602}M_{602}L_{602} and PSI–LHCI–LHCII mainly because higher orientation factor values in all LhcbM subunits.

The crystal structures of the vascular plant LHCII trimer have been resolved. Notably, the crystallized LHCII trimer has been reported to exhibit a photoprotective-dissipation state. Therefore, we compared the Chl configurations in the LHCII trimers amongst the crystallized LHCII trimer, LHCII trimers in PSII–LHCII supercomplexes (C_{602}S_{18} and C_{602}S_{19}), and PSI–LHCI–LHCII to examine the change(s) responsible for inducing the quenching state of LHCII (Figure S1A) (Table S2). Because each LHCII subunit of the plant LHCII trimer has not been completely identified, the respective LHCII subunits were compared based on the locations of the LHCII trimers in C. reinhardtii whereas LHCII subunits of crystallized LHCII trimers were not classified due to its uncertainty. The distance and orientation factor of each Chl pair in each LHCII subunit are shown in Figure S1B. In all LHCII subunits, Chl configurations varied in crystallized LHCII trimers and in different photosystem supercomplexes. Furthermore, symmetric LHCII subunits in the 3 LHCII trimers of the LHCII crystal were observed. We compared the interaction factors based on the FRET principle to estimate the effect of the change in Chl configurations in the LHCII trimers (Figure S1C). Interestingly, Chl a611-a612 pairs in the LHCII-i and LHCII-iii subunits of the crystallized LHCII trimer exhibited ~25% lower interaction than those of PSII–LHCII and PSI–LHCI–LHCII supercomplexes, whereas Chl a611-a612 pairs in LHCII-ii of both the crystallized LHCII and the PSI–LHCI–LHCII supercomplex exhibited ~25% lower interaction factors than those of the PSII–LHCII supercomplexes. In contrast, Chl b606-a604 pairs in the LHCII-i and LHCII-iii subunits of the crystallized LHCII trimer and the PSI–LHCI–LHCII supercomplex had a longer distance than those of the PSII–LHCII supercomplexes. For the LHCII-ii, the Chl b606-a604 pair in the crystallized LHCII trimer exhibited a longer distance than those of the PSII–LHCII and PSI–LHCI–LHCII supercomplexes. These results indicate that the Chl a611-a612 pair in LHCII-ii is analogous between the crystallized LHCII and the PSI–LHCI–LHCII supercomplex, whereas the Chl b606-a604 pair in the LHCII-i and LHCII-iii are analogous between the crystallized LHCII and the PSI–LHCI–LHCII supercomplex. These results indicate that
configurational change in the low-energy Chl cluster may be responsible for switching between the light-harvesting and photoprotective states of LHCII.

Discussion

Comparative analysis of the molecular structures of the photosystem supercomplexes revealed that the polypeptide structures and Chl coordinates of the LHCII trimer are flexible based on where the LHCII trimer binds. Quantitative comparisons of the configurational factors and estimation of the interaction factors between Chl pairs demonstrate that these flexible Chl configurations can significantly affect the energy transfer dynamics in LHCII. These results imply a regulation mechanism of the light-harvesting system by modulating protein-protein interactions, as reported previously for the array formation of PSII–LHCII supercomplexes and aggregation of the LHCII trimers. We expect that this mechanism enables green algae to modulate the light-harvesting system according to the environmental factors, such as intensity as well as quality of light, and the redox state of the electron acceptor pool.

Our comparative analysis on vascular plant LHCII trimers demonstrated that Chl configurations in the crystalized LHCII trimers, which has been known to be predominantly localized in this cluster. Moreover, this cluster is proposed to transfer the excitation energy to the excited state of the low-lying carotenoid, Lutein-1, which is in a photoprotective-dissipation state. We therefore propose that a configurational change in the low-energy Chl cluster switches the light-harvesting and photoprotective modes of LHCII. Our results demonstrated that the configurational factors of Chl a611-a612 and Chl a610-a612 are significantly altered, whereas those of Chl a610-a611 were not (Table 1). This result indicates that Chl a612 plays a pivotal role to control the excitation energy transfer dynamics in LHCII. Comparison of the LHCII subunits in the LHCII trimer also revealed that changes occur in outer LHCII subunits, LhcbM2 and LhcbM3, rather than in the inner LHCII subunit, LhcbM1. A decrease in interaction factors between Chl pairs implicate weaker coupling interactions resulting in a higher site energy than that of strong coupling interactions. This can ensure that the energy localization effect is smaller in outer LHCII subunits; therefore, it increases energy transfer efficiency to the core complexes in PSII C2S2M2L2 and PSI–LHCII–LHCII.

The other notable change was observed in the closest Chl pair b606-a604, which functions as an energy transmitter among the LHCII subunits in the LHCII trimer. Therefore, longer distances between the Chl pair b606-a604 can affect the inter-monomer energy transfer. We postulate that the inter-LHCII energy transfer from LhcbM2 to other LHCII subunits is inhibited such that external energy transfer from the S-LHCII trimer to M-LHCII trimer is promoted in C2S2M2L2, as previously proposed by Sheng et al. Therefore, we propose that the formation of C2S2M2L2 under low-light conditions increases the light-harvesting capability, and formation of C2S2M2L2 under high-light conditions suppresses the light-harvesting capability, thereby inhibiting over-excitation at the core complexes.

Our comparative analysis on vascular plant LHCII trimers demonstrated that Chl configurations in the crystalized LHCII trimers, which is regarded as photoprotective-dissipation state LHCII trimers, were distinct from that in PSII–LHCII and PSI–LHCII–LHCII supercomplexes. Notably, Chl a611-a612 and Chl b606-a604 pairs showed significant difference, which were the lowest energy site and an energy transmitter among the LHCII subunits in the LHCII trimers. These differences are supposed to lead changes in excitation energy transfer dynamics in the LHCII trimers. We regard these changes in Chl configurations act as a switch between light-harvesting and photoprotective dissipation states of the LHCII trimers. To prove it, further structural analysis to exclude the effect by the observation methods (cryo-EM vs. crystallography) to the conformational change and theoretical studies to investigate the energy transfer dynamics would be required.

Finally, we would like to highlight our findings about the molecular structures of photosystem supercomplexes. We propose that the light-harvesting efficiency by the LHCII trimer is higher in PSII–LHCII C2S2M2L2 and PSI–LHCII–LHCII supercomplexes but lower in PSII–LHCII C2S2 supercomplex due to the conformational changes of the LHCII trimer. In this study, we present relatively large structural differences rather than finer structural differences; however, it would be necessary to determine the precise Chl coordinates for further quantum mechanical analysis of energy transfer dynamics, which require higher spatial resolution of the structural data. Moreover, the possibility that the Chl coordinates in LHCII within the biochemical samples, such as supercomplexes, can be affected by detergents or buffer conditions during sample preparation. Although we compared LHCII trimers in the same buffer conditions in this study (Table S1), the effect of buffer conditions on the conformation and Chl configuration of the LHCII trimer should be studied.
carefully and systematically. The details of energy transfer dynamics remain to be studied by multiscale quantum mechanics/molecular mechanics (QM/MM) molecular dynamics simulations on these conformational changes in LHCII trimers.

Materials And Methods

Superposition and RMSD calculations using PDB data

Protein data bank (PDB) data listed in Table S1 and S2 were used in this study. Superposition of polypeptides structures and Chl a611-a612 pairs of LhcbM1 (6kac chain Y, 6kad chain Y, 7dz7 chain Z), LhcbM2 (6kac chain G, 6kad chain G, 7dz7 chain X), and LhcbM3 (6kac chain N, 6kad chain G, 7dz7 chain Y) were performed using the Pair fitting function of Pymol (PyMOL(TM) Molecular Graphics System, Version 1.8.6.0. Schrodinger, LLC.) with magnesium atoms and NA, NB, NC, ND atoms of the porphyrin rings of the Chls. Least square comparison of LHCII trimer structures and calculation of the root-mean-square deviations (RMSD) of the Cα atoms were performed by LAQKAB in CCP4. The color-coded LHCII structures for the RMSD values were represented by Pymol.

Computational analysis of configurational factors and FRET rate constants

In order to estimate the configurational factors, we applied a customized algorithm on the Python platform (Python v.3.6). As an interaction factor for the impact of configurational changes in Chl pairs, FRET rate constants were calculated based on FRET theory. The FRET rate constants ($k_{\text{FRET}}$) were defined as $k_{\text{FRET}} = (C\times \kappa^2)/(n^4R^6)$; where, $C$ is the factor calculated from spectral overlap integral between the two Chls, $\kappa^2$ is the dipole orientation factor, $n$ is the refractive index, and $R$ is the distance between two Chls. The applied $C$ values for Chl a→Chl a, Chl a→Chl b, Chl b→Chl a, and Chl b→Chl b were 32.26, 1.11, 9.61 and 14.45, respectively; these values were estimated by Gradinaru et al. $\kappa^2$ was defined as $\kappa^2 = \left[\hat{u}_D \cdot \hat{u}_A - 3(\hat{u}_D \cdot \hat{R}_{DA})(\hat{u}_A \cdot \hat{R}_{DA})\right]^2$, where $\hat{u}_D$ and $\hat{u}_A$ are the dipole unit vectors of donor and acceptor Chls derived using the vectors from the coordinates of NB and ND atoms, $\hat{R}_{DA}$ and is the unit vector of the vector originating from the magnesium of the donor Chl to the magnesium of the acceptor Chl. The value of $n$ was 1.55. $R$ was taken from the distance between central magnesium atoms of the two Chls. FRET rates were computationally calculated using a customized algorithm on the Python platform (Python v.3.6). For S-trimers of PSII–LHCII supercomplexes (6kac, 6kad, 3jcu, and 5xnl), configuration factors, and interaction factors from two S-trimers were determined. For crystallized LHCII trimers (1rwt), configuration factors and interaction factors from all three LHCII trimers were indicated.

Declarations

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Author contributions

E.K., H.K.-K, F.K., and J.M. conceived and designed the research. H.K.-K. and F.K. conducted comparison analyses of polypeptide structures, chlorophylls, and electron density maps. E.K. performed computational calculations for configurational factors and interaction factors between chlorophylls. E.K. curated the data and wrote the initial draft of the manuscript. J.M. provided supervision and revised the manuscript. All authors contributed to revising the manuscript and approve the final version of the manuscript.

Competing Interests Statement

The authors declare no competing interests.

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Figures

Figure 1

Conformations of LHCII trimers in three different photosystem supercomplexes. (A) LHCII trimer comprising LhcbM1, LhcbM2, and LhcbM3, and the locations of the LHCII trimers in PSII–LHCII supercomplexes—C2S2 (pdb.6kad) and C2S2M2L2 (pdb.6kad)—and a PSI–LHCI–LHCII supercomplex (pdb.7dz7) from a green alga C. reinhardtii. LhcbM1, LhcbM2, and LhcbM3 are represented by orange, purple, and yellow colors, respectively. (B) Superposed polypeptide structures and (C) superposed image of porphyrin rings of Chls in the LHCII trimers of the three different photosystem supercomplexes. LHCII trimers from C2S2, C2S2M2L2, and PSI–LHCI–LHCII supercomplexes are represented by pink, green, and cyan colors, respectively.
Figure 2

Conformational differences between the LHCII trimers. (A) Polypeptide structures of LHCII subunits, color-coded for the root mean square deviation (RMSD) values from $C_2S_2$ vs. $C_2S_2M_2L_2$, $C_2S_2M_2L_2$ vs. PSI–LHCI–LHCII, and PSI–LHCI–LHCII vs. $C_2S_2$. RMSD values are indicated with colors ranging from blue (identical) to yellow (different, >2.0Å). (B) Two different side-views of the LhcbM2-LhcbM1, color-coded for the RMSD values from $C_2S_2M_2L_2$ vs. PSI–LHCI–LHCII, with placements of Chls and carotenoids.
Relative configurations of Chl a611 and a612 and their cryo-EM maps. Chl a611 was overlayed to compare relative configuration of Chl a612 using Mg, NA, NB, NC, and ND atoms in its porphyrin rings. Both porphyrin rings of the Chls and cryo-EM maps of C₂S₂, C₂S₂M₂L₂, and PSI–LHCI–LHCII are represented by pink, green, and purple colors, respectively.
Figure 4

Configurational factors of Chls in LHCII trimers. (A) Scatter plots of configuration factors (Orientation factor and distance) between all Chls in each LhcbM subunit of each photosystem supercomplex. (B) Scatter plots of interaction factor (Energy transfer rate based on the FRET principle) and 1/distance^6 derived from (A). (C) Scatter plots of the configuration factors between Chls mediating inter-LHCII energy transfer (LhcbM1-LhcbM2, bM1-bM2; LhcbM1-LhcbM3, bM1-bM3; LhcbM2-LhcbM3, bM2-bM3) in the LHCII trimer of each photosystem supercomplex. (D) Scatter plots of interaction factors and 1/distance^6 derived from (A). C_2S_2, C_2S_2M_2L_2, and PSI–LHCl–LHCII are represented by pink, green, and cyan colors, respectively.

Supplementary Files

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