Energy transfer of aromatic amino acids in photosystem 2 core antenna complexes CP43 and CP47

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Abstract

Energy transfer of aromatic amino acids in photosystem 2 (PS2) core antenna complexes CP43 and CP47 was studied using absorption spectroscopy, fluorescence spectroscopy, and the 0.35 nm crystal structure of PS2 core complex. The energy of tyrosines (Tyrs) was not effectively transferred to tryptophans (Trps) in CP43 and CP47. The fluorescence emission spectrum of CP43 and CP47 by excitation at 280 nm should be a superposition of the Tyr and Trp fluorescence emission spectra. The aromatic amino acids in CP43 and CP47 could transfer their energy to chlorophyll (Chl) *a* molecules by the Dexter mechanism and the Föster mechanism, and the energy transfer efficiency in CP47 was much higher than that in CP43. In CP47 the Föster mechanism must be the dominant energy transfer mechanism between aromatic amino acids and Chl *a* molecules, whereas in CP43 the Dexter mechanism must be the dominant one. Hence solar ultraviolet radiation brings not only damages but also benefits to plants.

Additional key words: absorption; β-carotene; chlorophyll; fluorescence; model; proteins; tryptophan; tyrosine.

Introduction

CP43 and CP47, the core antenna subunits of photosystem 2 (PS2), are encoded by the *psb*C and *psb*B genes, respectively, in the chloroplast genome of higher plants and green algae and in the genomic DNA of cyanobacteria. The main function of CP43 and CP47 is to accept excitation energy that is harvested by the light-harvesting complex 2 (LHC2) and then transfer it directly to the PS2 reaction centre (RC) (Bricker 1990, Barber *et al.* 2000, Bricker and Frankel 2002). The PS2 crystal structure shows that there are 14 chlorophyll (Chl) *a* and 4 β-carotene (β-Car) molecules in CP43 and 16 Chl *a* and 2 β-Car molecules in CP47. There are no Chl *b* molecules in CP43 or CP47 (Ferreira *et al.* 2004). Pigments in CP43 or CP47 are bound in an orderly manner to the transmembrane part of the protein. The native array of the pigments is the basis for energy transfer function in the antenna complex. So the energy transfer between pigments has been investigated explicitly (Alfonso *et al.* 1994, Groot *et al.* 1995, Wang *et al.* 1999, 2000, Shan *et al.* 2000, 2001, De Weerd *et al.* 2002, Guo *et al.* 2004). However, the energy transfer between aromatic amino acids themselves, and the energy transfer between aromatic amino acids and pigments in CP43 and CP47 have not been investigated yet.

CP43 and CP47 have 473 and 510 amino residues, respectively, and both of them have six transmembrane α-helices, which are separated by five extrinsic loop domains (Bricker and Frankel 2002). Aromatic amino acids in protein can absorb ultraviolet (UV) radiation. Tryptophan (Trp) and tyrosine (Tyr) are the two main kinds of aromatic amino acid that relate to the absorption and fluorescence of protein in the UV region. For CP43, there are 5 Trps and 3 Tyrs in the transmembrane domains, and 12 Trps and 9 Tyrs in the extramembrane domains. For CP47, there are 5 Trps and 1 Tyr in the transmembrane domains, and 10 Trps and 16 Tyrs in the extramembrane domains (Barber *et al.* 2000, Ferreira *et al.* 2004). Aromatic amino acids in bacteriorhodopsin can transfer their energy to chromophores close to them (Kalisky *et al.* 1981, Henderson *et al.* 1990). In the last few decades, depletion of the stratospheric ozone layer has resulted in an increased transmission of solar ultraviolet (UV) radiation to the Earth's surface (Kalbin *et al.* 2005). Therefore, studying the UV absorption and transfer by aromatic

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Abbreviations: Chl – chlorophyll; GuHCl – guanidine hydrochloride; LHC2 – light-harvesting complex 2; PS – photosystem; RC – reaction centre; Trp – tryptophan; Tyr – tyrosine; UV – ultraviolet; β-Car – β-carotene.
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amino acids in light-harvesting proteins of plants is of great importance. We studied the energy transfer of aromatic amino acids in CP43 and CP47 using absorption spectra, fluorescence spectra of the native CP43 and CP47, and their changes induced by guanidine hydrochloride (GuHCl). In addition, the 0.35 nm crystal structure of the PS2 core complex (Ferreira *et al.* 2004) was also used to probe this problem.

Materials and methods

Purification of CP43 and CP47: PS2-enriched membranes were prepared from spinach as described in Kuwabara and Murata (1982). The oxygen-evolving core complex was isolated as described in Ghanotakis *et al.* (1989). CP43 and CP47 were then purified according to the method in Alfonso *et al*. (1994) with some modifications (Shan *et al.* 2001). CP43 and CP47 were judged to be pure on the basis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1).

GuHCl treatment: The purified CP43 or CP47 was suspended in 20 mM Bis–Tris and 0.05 % dodecyl maltoside (DM) (pH 6.0). The final concentrations of Chl *a* in the CP43 and CP47 preparations were 3 g m^{-3}. In a preliminary experiment we found that no further changes were observed when the treatment time exceeded 6 h or the GuHCl concentration exceeded 7 M. Therefore, we treated in this study CP43 and CP47 with 0–7 M GuHCl in the dark at room temperature for 6 h.

Spectral measurements: The absorption spectra were recorded with a *UV-3000* spectrophotometer (*Hitachi*, Tokyo, Japan) at room temperature. Fluorescence spectra were measured by an *F-4500* fluorescence spectrophotometer (*Hitachi*, Tokyo, Japan). Both the excitation and emission band passes were 5.0 nm, and the scan rate was 120 nm min^{-1} with a time constant of 2 s.

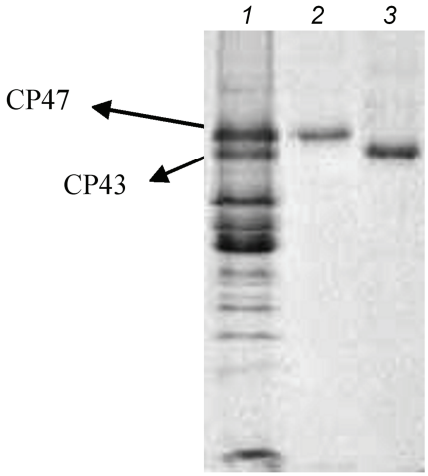

Fig. 1. Electrophoretic pattern of purified CP43 and CP47 in 15 % acrylamide gel containing 6 M urea. Lanes *1*, *2*, and *3* are for photosystem 2, CP47, and CP43, respectively. Proteins were stained with Coomassie brilliant blue R-250.

Results and discussion

Energy transfer from Tyr to Trp molecule in CP43 and CP47: The UV emission spectra of CP43 and CP47 are due to the constituent aromatic amino acids, Trp and Tyr (Burstein *et al.* 1973). Irradiation with 280 nm can excite both Trp and Tyr residues, whereas that with 295 nm excites exclusively the Trp residue (Eftink and Shastry 1997, Shutova *et al.* 2001). Proteins lacking Trp will only show Tyr fluorescence when excited at 280 nm, which occurs at about 304 nm and is insensitive to solvent. In contrast, the fluorescence of general proteins with both Trp and Tyr will be dominated by the contribution from Trp residues when excited at 280 nm. This is because Trp has a higher molar extinction coefficient and fluorescence quantum yield than Tyr and can serve as an energy transfer acceptor for Tyr (Oikawa *et al.* 1985, Barrow *et al.* 1992, Eftink and Shastry 1997).

Fig. 2 shows that upon excitation at 280 nm, the fluorescence emission maxima of native CP43 and CP47 were at 323 and 324 nm, respectively. Upon excitation at 295 nm, the two fluorescence emission maxima of native CP43 were at 324 and 346 nm, whereas those of native CP47 were at 321 and 339 nm, respectively. For many water-soluble proteins, the energy absorbed by Tyr can be effectively transferred to Trp, so the emission spectra when excited at 280 or 295 nm are identical (Lakowicz 1983, Oikawa *et al.* 1985, Eftink and Shastry 1997, Isaev-Ivanov *et al.* 2000, Dubey and Jagannadham 2003). Unlike the water-soluble proteins, upon excitation at 280 and 295 nm the emission spectra of native CP43 or CP47 were not identical (Fig. 2). Thus, the energy of Tyr was probably not effectively transferred to Trp. The maxima at 323 nm for CP43 or 324 nm for CP47 should be a superposition of Tyr and Trp emission, which is similar to the results reported for other membrane proteins (Lakowicz 1983, Shutova *et al.* 2001).

Energy transfer from aromatic amino acids to Chl *a* molecules in CP43 and CP47: Changes of aromatic amino acid fluorescence can reflect the changes of protein tertiary structure and so they provide a valuable structural probe for the study of protein unfolding (Kelly and Price 1997, Bian *et al.* 2000, Tanaka *et al.* 2002). Basically, with the unfolding of a protein, the Tyr and Trp residues in the inner low-polarity regions would be

exposed to a polar environment gradually, and the fluorescence emission maximum would suffer a red shift (Dubey and Jagannadham 2003). GuHCl is the most powerful among commonly used protein denaturants (Nozaki 1972), and it offers many advantages over other means of unfolding protein such as acid, heat, or detergent (Pace 1986). After GuHCl treatment, the native proteins of CP43 and CP47 were unfolded gradually with the increasing GuHCl concentration, as could be seen from the red-shifted fluorescence emission maximum when excited at 280 or 295 nm (Fig. 2). When treated with 7 M GuHCl, the emission maxima of both CP43 and CP47 were at 354 nm (Fig. 2). This indicated that CP43 and CP47 were denatured completely because 354 nm is the emission maximum of Trp when completely exposed to water (Burstein *et al.* 1973, Eftink and Shastry 1997, Isaev-Ivanov *et al.* 2000). In addition, after 7 M GuHCl treatment no pigments were dissociated from CP43 and CP47, which was verified by ultracentrifugation.

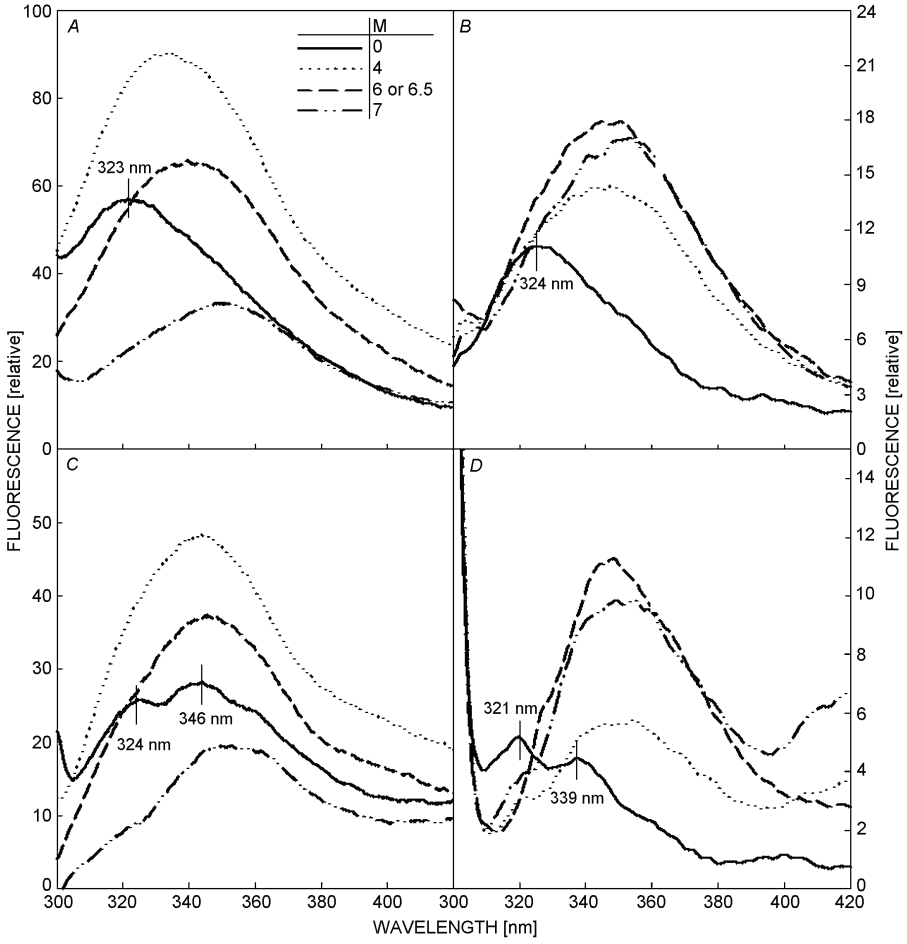

Fig. 2. Effects of GuHCl on the fluorescence emission spectra of CP43 (*A, C*) and CP47 (*B, D*) excited at 280 nm (*A, B*) and 295 nm (*C, D*). The spectra were measured after samples were incubated in solutions with different concentrations of GuHCl (the third one in *A* and *C* was 6 M, in *B* and *D* 6.5 M) in the dark at room temperature (25 °C) for 6 h. The final concentration of Chl *a* in the CP43 and CP47 preparations was 3 g m^{-3}.

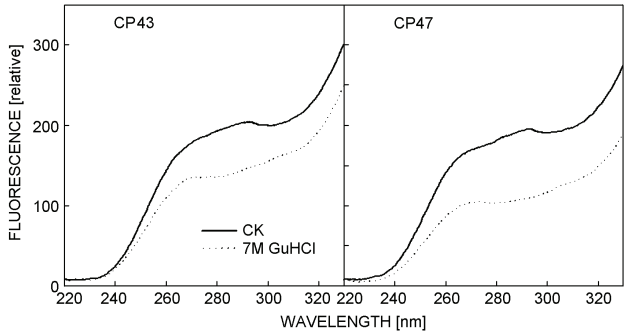

Fig. 3. Effects of 7 M GuHCl on fluorescence excitation spectra of CP43 and CP47 in the UV region. The detection wavelength was the emission maximum of Chl *a* in CP43 and CP47. See legend of Fig. 2.

Fig. 3 shows that the intensity of excitation spectra of the native CP43 and CP47 was higher than that of the denatured CP43 and CP47 in the UV region. Similar to aromatic amino acids, Chl *a* absorbs in the UV region. In the native CP43 or CP47, some Chl *a* molecules are very near to the aromatic amino acids. So it is proposed that aromatic amino acids transfer their energy to Chl *a* molecules around them. As a result, the intensity of excitation spectra of Chl *a* in the UV region did increase. When treated with 7 M GuHCl, the apoproteins of CP43 and CP47 were denatured completely, so the distance between Chl *a* molecules and aromatic amino acids was increased and then the energy transfer between them disappeared. As a result, the intensity of excitation spectra of Chl *a* in the UV region was decreased. In all,

the difference of the intensity of excitation spectra between the native and the denatured CP43 or CP47 proves that aromatic amino acids can transfer their energy to Chl *a* molecules.

The energy transfer efficiency from aromatic amino acid to Chl *a* can be estimated by $(F_n - F_d)/F_n \times 100$ %, where F_n is the intensity of excitation spectrum of the native protein at a certain excitation wavelength, and F_d is the intensity of excitation spectrum of the protein denatured by 7 M GuHCl at the same excitation wavelength. By calculation of the data in Fig. 3, the energy transfer efficiency was 30 % for CP43 and 42 % for CP47 when excited at 280 nm, while it was 26 % for CP43 and 43 % for CP47 when excited at 295 nm. These results show that the energy transfer efficiency from aromatic amino acid to Chl *a* in CP47 was much higher than that in CP43.

When treated with GuHCl, the intrinsic fluorescence intensities of CP43 and CP47 both increased at first but decreased later with the increasing GuHCl concentration (Fig. 2). Generally, the increase may be the result of increased distance between Trp and specific quenching groups, such as disulfide, protonated histidine, peptide bonds, certain metal ions, heme groups, and so on (Eftink and Shastry 1997, Dubey and Jagannadham 2003). Unlike CP43 and CP47, the fluorescence intensity of general water-soluble proteins will only decrease when unfolded by different concentrations of GuHCl (Dubey and Jagannadham 2003). The main difference between general water-soluble proteins and CP43 and CP47 is that CP43 and CP47 are membrane proteins with pigments. Therefore, we suggest that the most important quenching group in CP43 and CP47 must be Chl *a*. After GuHCl treatment, the distance between aromatic amino acids and Chl *a* molecules increased; thus, the quenching function of Chl *a*, namely the energy transfer from aromatic amino acids to Chl *a* molecules decreased, and then the intrinsic fluorescence intensity of aromatic amino acids was increased. In conclusion, after GuHCl treatment the increase of intrinsic fluorescence intensity of CP43 and CP47 also proves that that excitation energy can be transferred from aromatic amino acids to Chl *a* molecules.

Upon excitation at 280 or 295 nm, the fluorescence intensity of CP43 greatly exceeded that of CP47 (Fig. 2), even though their Trp and Tyr amounts did not differ too much (Barber *et al.* 2000). This implied that the quenching function of Chl *a* in CP47 must be greater than that in CP43. In other words, this also proved that the excitation energy transfer efficiency from aromatic amino acid to Chl *a* in CP47 was much higher than that in CP43. This result is similar to the calculated result shown above.

Two energy transfer mechanisms between aromatic amino acids and Chl *a* molecules in the native CP43 and CP47: Energy transfer occurs by Dexter mechanism with a measurable efficiency if the edge-to-edge distance between chromophores is less than 0.5 nm (Barber *et al.* 2000). Within the Ferreira PS2 crystal structure (Ferreira *et al.* 2004) there are 11 Trps and 4 Tyrs whose edge-to-edge distance to Chl *a* is less than 0.5 nm in CP43. In CP47, that is 7 Trps and 3 Tyrs (Table 1). So, the Dexter mechanism must be an efficient energy transfer mechanism between aromatic amino acids and Chl *a* molecules in the native CP43 and CP47. In addition, based on the Föster mechanism, the excitation energy transfer can occur if the fluorescence emission spectrum of a chromophore overlaps with the absorption spectrum of another chromophore, and the distance between them falls within the range of ~1–10 nm (Föster 1965). Figs. 2 and 4 show that the emission spectrum of Trp and Tyr overlaps much with the absorption spectrum of free Chl *a*. In addition, the Ferreira PS2 crystal structure (Ferreira *et al.* 2004) shows that there are many Trps and Tyrs whose distance to Chl *a* falls within the range of ~1–10 nm. So, the Föster mechanism must be another efficient energy transfer mechanism between aromatic amino acids and Chl *a* molecules in the native CP43 and CP47.

Table 1. The aromatic amino acids whose edge-to-edge distances to Chl *a* molecules around them were less than 0.5 nm in CP43 and CP47. The corresponding Chl *a* molecules were also described. Data were obtained from the crystal structure of the photosystem 2 core complex (Ferreira *et al.* 2004) deposited in RCSB PDB, entry 1S5L.

CP43		CP47	
Aromatic amino acid	Chl *a*	Aromatic amino acid	Chl *a*
Trp63	12, 13, 17,18	Trp33	26, 24, 30, 28
Trp36	20, 44	Trp 91	27
Trp151	15	Trp115	32
Trp239	18	Trp185	33, 46
Trp250	11	Trp450	26
Trp259	11	Trp468	35
Trp266	11, 22	Tyr6	32,35
Trp425	12	Tyr40	26
Trp443	20	Tyr117	37
Tyr131	21		
Tyr143	21		
Tyr274	14, 22		
Tyr297	16		

Although there are less aromatic amino acids whose distance to Chl *a* is smaller than 0.5 nm in CP47 than in CP43 (Table 1), the energy transfer between molecules of aromatic amino acids and Chl *a* in CP47 is greater than that in CP43. This implies that in CP47 the Föster mechanism must be the dominant energy transfer mechanism between aromatic amino acids and Chl *a* molecules, whereas in CP43 the Dexter mechanism must be the dominant one.

In CP43 and CP47, there are also β-Car molecules in addition to Chl *a* molecules. However, there are no

aromatic amino acids whose edge-to-edge distance to β-Car is less than 0.5 nm (Ferreira *et al.* 2004), so it is impossible for aromatic amino acids to transfer their energy to β-Car molecules by the Dexter mechanism. In addition, although the emission spectrum of aromatic amino acids overlaps with the absorption spectrum of β-Car in CP43 and CP47, the absorbance of β-Car in the UV region is very weak (Figs. 2 and 4), so the energy transfer from aromatic amino acids to β-Car molecules by the Föster mechanism in the native CP43 and CP47 should be limited.

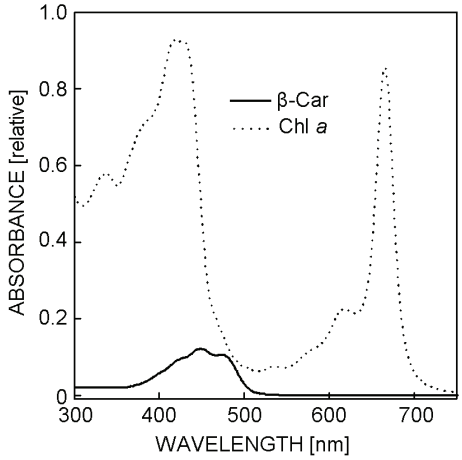

Fig. 4. The absorption spectra of free chlorophyll (Chl) *a* and β-carotene (β-Car) that were suspended in 20 mM Bis–Tris and 0.05 % DM (pH 6.0). The ratio Chl *a*/β-Car resembled that in the native CP43 and CP47.

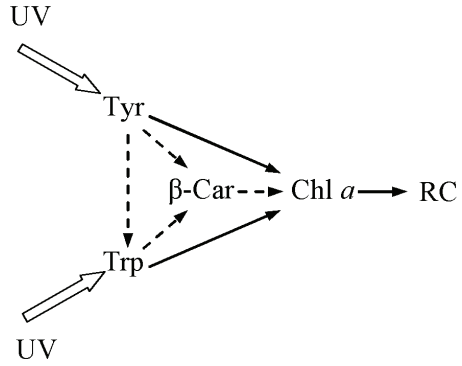

Fig. 5. Model of excitation energy transfer pathways between aromatic amino acids and reaction centre (RC). ⇨ denotes the energy absorbed by aromatic amino acids. ——► denotes the major pathway, ------► the minor pathway. Chl – chlorophyll, β-Car – β-carotene.

Based on the above discussion, the energy transfer pathways from aromatic amino acids in CP43 or CP47 to the RC can be summarized as shown in Fig. 5. As a general rule, the solar UV is harmful to plants, which has been explicated in many articles. Nevertheless, we found

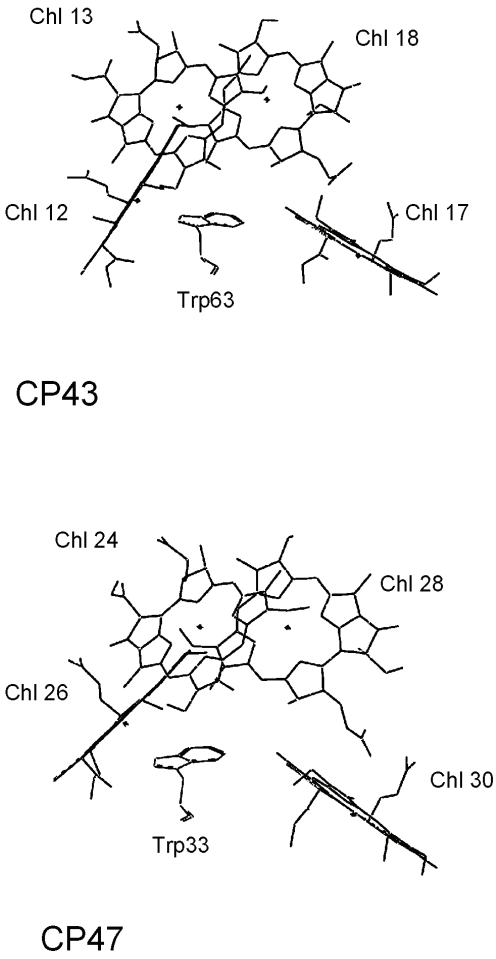

Fig. 6. Positions of Trp 63 in the native CP43 and Trp 33 in the native CP47 relative to the 4 chlorophyll (Chl) *a* molecules close to them. Data were obtained from the crystal structure of the photosystem 2 core complex (Ferreira *et al.* 2004) deposited in RCSB PDB, entry 1S5L.

that the solar UV radiation has also some benefits to plants.

Two special aromatic amino acids in the native CP43 and CP47: In the native CP43, Trp 63 is the only Trp whose distance to 4 Chl *a* molecules is less than 0.5 nm simultaneously. In the native CP47 the similar Trp is Trp 33 (Table 1). Both of those two Trps are the third amino acid of the first α-helix in CP43 and CP47. The positions of Trp 63 and Trp 33 relative to the 4 Chl *a* molecules close to them are also very similar (Fig. 6). In all, Trp 63 in CP43 and Trp 33 in CP47 manifest high conservation in their position. Considering the energy transfer function and high hydrophobic property of Trp in CP43 and CP47, it is proposed that these two Trps might be important in energy transfer and maintain the structural integrity of the native CP43 and CP47. Whether this is true or not remains to be studied further.

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