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Reconstitution Approach to Tune Spectral Features of Light Harvesting Complexes for Improved Light Absorption and Energy Transfer

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Abstract

Light harvesting complexes developed by living organisms render themselves as an excellent system for understanding basic physical and chemical processes behind the conversion of sunlight energy. Although light harvesting complexes are pretty robust, biochemical reconstitution and genetic modifications have proven the flexibility to tailor their absorption spectra and energy transfer. Importantly, the refolding of the protein and the exchanging of the pigment in micellar media results in very similar pigment arrangement within the native complexes. Here, we show reconstitution approaches with different pigments that have been carried out in PCP, LHCII, and LHI complexes. Monitoring on the spectral changes and energy transfer has also been described.

Keywords: reconstitution methods, pigment-protein, light harvesting complex, light absorption, energy transfer

BCHL	Bacteriochlorophyll
β-OG	n-octyl beta-D-glucopyranoside
B780	Monomeric LH1 subunit
B820	Dimeric LH1 Subunit

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B870	Carotenoid-less LH1 complex
B880	Fully reconstituted LH1 complex
CD	Circular dichroism
CHL	Chlorophyll
CRT	Carotenoid
DEAE	Diethylaminoethanol
DGDG	Digalactosyldiacylglycerol
iB873	Intermediate complex in the assembly of LH1 complex
LDAO	N,N-dimethyldodecylamine N-oxide
LDS-PAGE	Lithium dodecylsulfate polyacrylamide gel electrophoresis
LH	Light harvesting
LHC	Light harvesting complex
NEU	Neurosporene
NIR	Near infrared
Ni-BCHL	Ni-substituted bacteriochlorophyll a
N-PCP	Apoprotein of N-terminal domain from PCP
PCP	Peridinin chlorophyll protein
RB	Rhodobacter
RC	Reaction center
RSP	Rhodospirillum
SDS	Sodium dodecylsulfate
SPH	Spheroidene

1. Introduction

Sun is one of the most attractive sources of alternative energy. The total energy of sunlight that irradiates the surface of the Earth is believed to provide sufficiently the requirement of a clean and green energy of human population [1]. It will become profitable when a few per cent of this energy can be collected and transformed into some usable energy. Photovoltaic is one of the current technologies that are able to transform sunlight into electricity. The photovoltaic effect was discovered as early as in the first half of the 19th century by Becquerel. Its physical principle, i.e. a light-driven charge separation, resembles the one followed by natural photosynthetic system [2].

Enormous progress in determining the structure of photosynthetic pigment-protein complexes has been carried out using x-ray crystallography to understand the exact position, orientation of the pigments with the resolution $\leqslant$ 2.5Å as well as their binding within the protein matrix [3–5]. In conjunction with spectroscopic data, it reveals how the light harvesting complexes (LHCs) are functioning to capture the energy efficiently and transfer it to the reaction centers (RCs) and how the charge separation occurs in the latter [6]. The structures of LHCs show large variation in terms of the pigment composition depending on the available light spectrum for certain ecological niche in which that photosynthetic organism lives. In the higher plants, chlorophyll (Chl) a plays important role in the light harvesting (LH) and other pigments, i.e. Chl *b* and carotenoids (Crts) act as accessory LH pigments. In dinoflagellate, most of the available light spectrum is in the blue-green region of the seawater column. Dinoflagellate have evolved LHCs in which most of the light capturing capacity is provided by Crts, which absorb maximally in the blue-green region of the spectrum. Similar situation occurs in the purple photosynthetic bacteria, which are found in the anaerobic layers of ponds pre-filtered by Chl-based photosynthetic organisms. Thus they use bacteriochlorophyll (BChl) for absorbing the remaining near-infrared (NIR) region of the light spectrum and Crts for absorbing the green light.

LH is the major function of Crt besides its photo-protecting function in photosynthesis process [7]. Crt acts as an accessory pigment to capture light in 400-550 nm spectral region and to transfer the energy to B(Chl). Crt serves as efficient energy donor to B(Chl) in the LH process due to a close contact of Crt with B(Chl) molecules in photosynthetic antenna. Moreover, the fluorescence from the first (S_1) and second (S_2) singlet exited states of Crt

has a good spectral overlap with Qy and Qx bands of B(Chl) which favors energy transfer. Therefore, the efficiency of intra-complex Crt to B(Chl) singlet energy transfer might be affected by densely packed pigments and the Crt structure in LHCs [8]. In dinoflagellate, peridinin as the main Crt and LH pigment in peridinin-chlorophyll-protein (PCP) has a high efficiency of energy transfer to Chl a, which approaches to 100%. 80-100% of the overall energy transfer efficiency from Crts to Chls in LHCII might depend on the exact Crt composition in green plant antenna proteins [9–11]. In purple photosynthetic bacteria, the structure of a particular Crt affects its ability to perform energy transfer to BChl *a*. The efficiencies of Crt to BChl energy transfer have been reported to be 80-100% for LH2 of *Rhodobacter sphaeroides* G1C containing mainly neurosporene (Neu) and LH2 of *Rb. sphaeroides* 2.4.1 containing mainly spheroidene (Sph) [12–15], 35-75% for LH2 of *Rhodospirillum acidophila* containing mainly rhodopin glucoside [14–17] and 30% for LH1 of *Rsp. rubrum* containing mainly spirilloxanthin [18].

The utilization of LHCs for applications in different environments triggers ways to modify its structure. Reconstitution of LHCs has been developed to exchange pigments with other types of pigments that have different absorption properties. This approach may be useful in increasing the capacity of LH, e.g. by expanding the range of absorption and enhancing the efficiency of intra-complex energy transfer. The main purpose of this review is to present exemplary reconstitutions and their progress prior to enhance harnessing capacity of solar energy. In this review, we focus on the works that have been done with the PCP from dinoflagellate, the major antenna complexes from purple photosynthetic bacteria (LH1) and from higher plants (LHCII).

2. Reconstitution in peridinin-chlorophyll protein (PCP)

Instead of collecting light mainly by Chls or BChls, the main LH pigment of PCP is peridinin, which absorbs light between 350 and 550 nm. The structure of PCP monomer from *Amphidinium carterae* consists of two Chl *a* molecules and eight peridinin molecules organized in two homologous clusters, which are embedded in protein matrix [4]. The center-to-center distance between Chl *a* is 17.4 Å. The resulting weak coupling between the two Chls and the high Crt-Chl ratio of 4:1 indicate a particular strategy for LH. Upon absorption of light by the peridinin, the energy is then transferred to Chl *a* with a quantum efficiency close to 100% [19, 20]. The energy transfer between the two Chl *a* can be described in terms of Förster energy transfer, with a transfer rate of 0.082 ps-1 [21].

PCP can be reconstituted with Chl *a*, Chl *b*, Chl *d*, acetyl-Chl *a*, and BChl *a* [22–25]. In this way the absorption in the region of NIR can be varied. Importantly, the reconstitution results in very similar pigment arrangement as with the native PCP [25]. PCP can also be reconstituted with Chl mixtures, e.g. Chl *a* and Chl *b* (N-PCP-Chl *a/b*); Chl *a* and acetyl-Chl *a* (N-PCP-Chl *a*/acetyl-Chl *a*) or Chl *b* and acetyl-Chl *a* (N-PCP Chl *a*/acetyl-Chl *a*) [24]. The reconstitution experiment requires apoprotein of N-terminal domain from PCP (N-PCP). It is usually composed of 150 amino acid residues and is topologically a "half-mer" of the monomeric native PCP protomer from *A. carterae* [22]. For the use of reconstitution N-PCP can be prepared by heterologous overexpression in bacteria [22, 26]. The reconstitution procedure was accomplished by mixing apoprotein and ethanol solution of pigments in 1:1.5 molar ratio followed by 48 h of incubation at 4°C [24, 26]. A monomer of PCP from A. carterae has one pair of lipid digalactosyldiacylglycerol (DGDG), which binds tightly to pigments [4]. The isolated pigments from native PCP from A. carterae already contain DGDG [25]. The use of pigments from different source can be suggested with an addition of DGDG to reconstitute "high-grade" of PCP for crystallization purpose [25].

The reconstitution procedure yields dimeric aggregates that correspond topologically to native monomer of PCP. The success of reconstitution can be monitored by spectroscopy methods, e.g. absorption, circular dichroism (CD), and fluorescence. Fig. 1 shows the results of reconstitution with different types of the Chl molecule. In these absorption spectra the incorporated Chls give Qy band centered at 670 nm (Chl *a*), 648 nm (Chl *b*), and 650 and 668 nm (Chl *a/b*). The visible region (400-600 nm) of the absorption spectra of PCP reconstituted with Chl *a* is similar to those of native PCP. Reconstitution with Chl *b* yields more pronounced Soret band in the visible region due to the larger extinction coefficient of Chl *b* as compared to Chl *a* [27]. The fluorescence spectrum of N-PCP-Chl a shows a single line at 673 nm originating from the S_1-S_0 transition of Chl *a* that has been generated by energy transfer from peridinin through excitation at 532 nm. In N-PCP-Chl *b*, the fluorescence emission is blue shifted to 652 nm. It was calculated that the fluorescence quantum yield of Chl *a* and *b* in N-PCP were 24% and 11%, respectively [28].
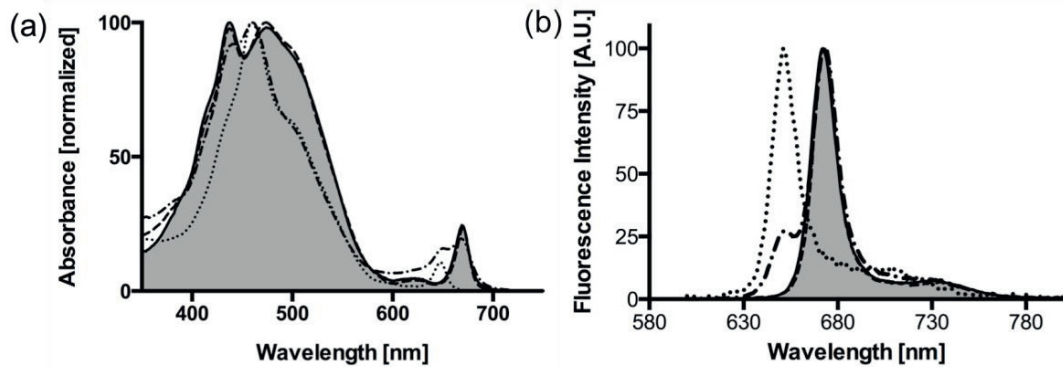
Figure 1. The absorption (a) and fluorescence emission (b) spectra of native PCP (black line with shaded area) and the reconstituted complexes: N-PCP Chl *a* (dashed line), N-PCP Chl *b* (dotted line), and N-PCP Chl *a/b* (dash-dotted line). The fluorescence emission spectra were taken with excitation wavelength at 532 nm.

The absorption spectra of the reconstituted PCP with Chl mixtures show superficially a mix of the spectra of reconstituted complexes with single type of Chl. The fluorescence spectrum of N-PCP reconstituted with both Chl *a* and b is also characterized by two emission lines corresponding to the two Chl pigments (Fig. 1B). The presence of reconstituted PCP containing Chl *a* in the one half and Chl *b* in the other half of dimeric aggregate can be monitored by single molecule spectroscopy [29]. The detailed experiment using single molecule spectroscopy can also show Förster energy transfer between Chl *a* and b in a monomer. The downhill energy transfer from Chl *b* to Chl *a* took about 31 ps, while the uphill energy transfer took 47 ps. It is also shown that energy transfer from peridinin to Chl *a* is almost three times more efficient than that from peridinin to Chl *b* [28].

3. Reconstitution in core light-harvesting complex II

An in vitro assembly of native and recombinant LHCIIb protein with its native chromophores has first been reported by Plumley and Schmidt [30] and Paulsen et al. [31], respectively. These methods rely on the self-assembly of sodium dodecylsulfate (SDS) denatured LHCIIb apoprotein when mixed with the total pigment extract of spinach leaves, followed by several freeze-thaw cycles. The minimum components required for LHCIIb folding were Chl *a/b* and xanthophylls, but not diacylglycerides.

A similar reconstitution technique was employed to assess the structure-function relationships in LHCII formed from its apoprotein overexpressed in bacterial hosts. The deletion mutagenesis has helped to elucidate the minimum amino acid requirements for LHCII assembly [32, 33]. Since the atomic model of LHCII provided by Kühlbrandt et al. [34] did not distinguish between Chl *a* and Chl *b*, the mutations of Chl-binding amino acid residues were expected to address the question of assignment of specific Chl species to their binding sites in the antenna [35, 36]. Surprisingly, partially inconsistent data have been obtained, leading the authors to the conclusion that some binding sites in LHCIIb are non-specific. This observation has been confirmed by Kleima et al. [37], when a wild-type LHCIIb was reconstituted with different Chl *a/b* ratios, ranging 1 Chl *a* per 35 Chl *b* molecules in LHCII trimer. Analysis of the results showed a large degree of promiscuity among most Chl *a* binding sites, which can be utilized to introduce Chls other than the native ones.

During over 25 years period, the protocol for LHCII reconstitution has been substantially improved. It relies on spontaneous refolding of SDS denatured LHCIIb in the presence of pigments dissolved in ethanol. More recently [38], the detergent exchange was achieved via mixed micelles formed in the presence of n-octyl beta-D-glucopyranoside (β-OG), and SDS is precipitated out at 4°C with potassium ions. After centrifugation, the supernatant yields monomeric LHCII and free pigments solubilized in the micelles. The latter can be efficiently separated from the complexes via partially denaturing polyacrylamide gel electrophoresis in the presence of lithium

dodecylsulfate (LDS-PAGE) [30–33, 36] or a combination of chromatographic and density gradient centrifugation methods [35, 37–39]. The assessment of preparation quality is based on the electronic absorption, CD and fluorescence emission measurements, which can be performed in excised green gel bands or after complex elution from the gel. Spectral properties of native and reconstituted LHCII, as presented in Fig. 2, are fairly similar [31, 38, 40]. The differences seen between two types of complexes, particularly in the CD spectra, can be explained by specific interactions between chromophores of individual LHCII monomers in the native antenna. In turn, the discrepancy in the Chl absorption intensities originates from altered Chl *a/b* ratio in the reconstituted LHCII (not shown), caused probably by promiscuity of Chl a binding sites [37].

LHCII reconstitution served to study the kinetics of antenna folding, thermodynamics of cofactor binding, and excitation transfer dynamics, but in contrast to LH1, very few reports can be found on pigment exchange in LHCII. Some modification of spectral properties has been introduced, e.g. by means of covalent attachment of rhodamine red to LHCIIb apoprotein, which did not interfere with complex assembly and functioning [39]. The efficient excitation energy transfer from the rhodamine molecules to Chls resulted in an increase in the absorption cross section and overall LH efficiency, but such an approach can not be considered as pigment exchange. Thus, LHCII and LH2 remain the antenna complexes whose pigment content and spectral features have not been modified by means of reconstitution. This fact is noteworthy, especially considering a large number of reports on other aspects of LHCII reconstitution, in contrast to LH2. The methods for pigment exchange in this complex are yet to be worked out.
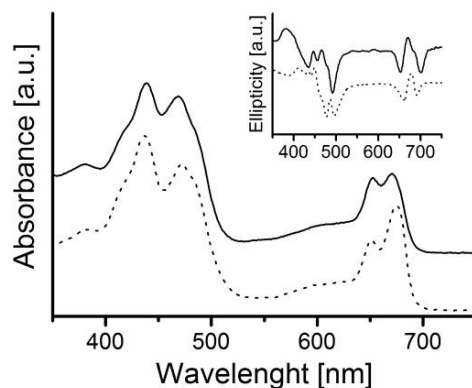

Figure 2. Electronic absorption and circular dichroism spectra of native trimeric (dotted line) and reconstituted monomeric (solid line) forms of LHCII. The samples were obtained according to [38], purified by LDS-PAGE method and eluted from the gel prior to the measurement.

4. Reconstitution in core light-harvesting I complex

The LH1 complex is the simplest type of photosynthetic antenna complexes which can now be isolated and is well characterized. In addition, several methods exist for its in vitro reconstitution from the individual components, which also provide means for pigment exchange [41, 42]. Reconstitution of LH1 proved highly successful approach to understand the structure-function relationships in this antenna [43]. According to the structural modeling and low resolution crystallographic data [44–46], the reaction center complex (RC) fits into the inner space of the LH1 complex. The two complexes closely interact to ensure a high efficiency of inter-complex energy transfer. LH1 is composed of 15-16 heterodimeric subunits of highly hydrophobic α- and β-polypeptides, which host a circular array of 30-32 strongly interacting BChl molecules and 15-16 Crt molecules. Both BChl and Crts are involved in the LH process. BChls are in contact with Crts via van der Waals interaction with the long alcohol residues esterifying the C-173 propionic acid side group [47].

The LH1 reconstitution was initially introduced by Paul A. Loach and the co-workers, taking advantage of the fact that LH1, in micellar media and in the absence of Crts, reversibly dissociates into dimeric B820 subunits and further to its monomeric B780 subunits. Prior to dissociation, Crts must be removed from LH1, either at the stage of

chromatophores of wild-type photosynthetic bacteria, e.g. by extraction with a hydrocarbon solvent, or using carotenoidless strains of bacteria. The Crt-depleted LH1 has the maximum absorption wavelength (λ_{max}) at around 870 nm, and is termed B870. On the basis of biochemical and spectroscopic properties, the composition of B820 subunit is assigned to $\alpha\beta\cdot2$BChl. Based on these properties, Paul A. Loach and the co-workers have used the native and modified BChls and the polypeptides for reconstituting the B820 subunits, B870 and LH1 complexes to reveal the structural requirements for their formation [48, 49]. These reconstitutions were performed on analytical scale and no attempts were made to isolate the reconstitution products.

The LH1 reconstitution approach has recently been further elaborated by Leszek Fiedor and the co-workers. The protocols developed provided means to obtain large quantities of the reconstituted pigment-protein complex. To this end, three different reconstitution strategies have been developed: (1) LH1 formation from organic solvent-extracted LH1 components, (2) LH1 formation from detergent-dissociated Crt-depleted antenna and (3) LH1 formation via oligomerization of the B820 subunit.

4.1 Reconstitution from organic solvent-extracted LH1 components

The components of LH1 from freeze-dried membrane of an LH1-only mutant of *Rb. sphaeroides* (DD13 strain) were extracted with a mixture of chloroform and methanol containing ammonium acetate [50, 51]. The extract, which contains pigments and polypeptides of the LH1 complex, was dried under vacuum. The resulted solid form was suspended in Tris-HCl buffer, followed by solubilization at high concentrations of β-OG (20% or 3.4%). The progress of reconstitution can be monitored as an increase in the absorption at 875 nm. The reconstituted LH1 complex is nearly identical to the native one in terms of absorption, fluorescence and CD spectra as well as energy transfer efficiency from Crt to BChl. Ion-exchange chromatography on a diethylaminoethanol (DEAE)-Sepharose column was used for purification of this reconstituted LH1 complex. Generally, the higher recovery of the reconstituted LH1 complex, up to 40%, is achieved in reconstitution with the lower detergent concentration (3.4%). This method allows for insertion of Ni-substituted analog of BChl (Ni-BChl) a to the LH1 complex, when this pigment is added at the stage of organic solvent extract.

4.2 Reconstitution from Crt-depleted antenna

A convenient way for reconstituting LH1 complex either from the Crt-depleted antenna of *Rsp. rubrum* or the Crt-less antenna of *Rsp. rubrum* G9, which facilitates Crt insertion, has been introduced by Fiedor et al. [42]. This reconstitution method can be applied for the insertion of modified BChl into LH1. The Crt-depleted antenna is prepared either from the freeze-dried chromatophores of *Rsp. rubrum* S1 after removal of Crts by benzene extraction or of the Crt-less strain *Rsp. rubrum* G9 [52]. The Crt-less antenna is solubilized in 0.03% N,N-dimethyldodecylamine N-oxide (LDAO) to achieve its dissociation into a mixture of the B780 and B820 subunits. Upon the addition of acetone, the equilibrium between the LH1 subunits shifts towards B870, due to an increase in dielectric permittivity of the medium [53]. The fully reconstituted LH1 complex (B880) can be formed in the presence of Crt added as acetone solution. The position of the Qy band of BChl a in this complex is red shifted by 10-12 nm compared to that in the Crt-less LH1. A similar shift is observed in the absorption of Crts. Most likely, excitonic coupling between BChl a molecules in LH1 is enhanced due to the Crts. Indeed, thermodynamic analysis of LH1 formation indicates strong interactions of Crts with the LH1 subunits [53]. Preparative purification of the reconstituted LH1-Crt complexes was performed by ion-exchange chromatography. If neccessary, a sucrose density gradient centrifugation step can be added to remove contaminating Crt aggregates [54]. The yields of the purified complexes with respect to the Crt-depleted antenna vary between 40% and 75%, depending on the stability of the complex [42, 54]. In order to introduce a modified BChl into LH1, e.g. Ni-BChl a, the addition of the pigment in acetone to the reconstitution mixture is done before the addition of Crt [55].

The spectral features of the Crt-less antenna and the LH1 complexes reconstituted with two Crts, Neu and Sph (Fig. 3), are in agreement with those reported previously [42, 53]. The Q_y band of BChl of the Crt-less antenna has λ_{max} at 870 nm, while this band of the reconstituted LH1-Crt complexes is typically red shifted to 880 nm due to the correct assembly of LH1 complex induced by Crt. Absorption spectra of the LH1 complexes reconstituted with Crts show that Crt absorption can cover the energy gap in blue-green region of BChl absorption spectrum to enhance the

efficiency of photon capturing. CD spectra of LH1 complex reconstituted with Crt show a specific feature of Crt absorption due to Crt being placed in the chiral environment of LH1 polypeptides. These results indicate the correct incorporation of Crt into the complex, which is also confirmed by a high efficiency of the intra-complex (Crt to BChl a) singlet energy transfer. The efficiency of this energy transfer decreases gradually from 80% to 30% along with increasing the number of conjugated double bonds (n) from 9 to 13. It has been shown that as the n in Crt increases above 11 not all low-lying singlet states of Crt are available as channels for energy transfer to BChl a [52, 54].
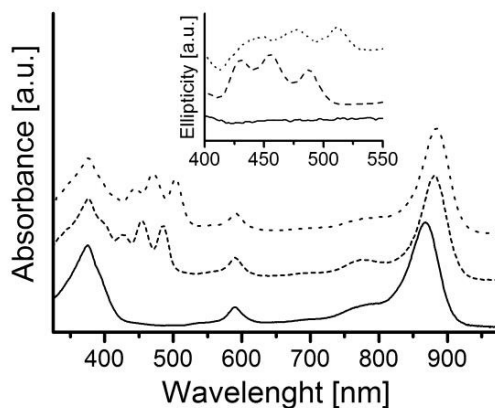

Figure 3. Electronic absorption and circular dichroism spectra of Crt-less antenna (B870, solid line) in 0.4% β-OG and the LH1 complexes reconstituted with neurosporene (LH1-Neu; dashed line) and spheroidene (LH1-Sph; dotted line) in 0.03% and 0.045% LDAO, respectively.

4.3 Reconstitution from the B820 subunit

The B820 subunit was prepared from the freeze-dried chromatophores of the Crt-less antenna of *Rsp. rubrum* by solubilization in 3.4% β-OG and purified on a DEAE-Sepharose column. The yield of pure B820 reaches 70-80% with respect to the initial amount of the chromatophores. LH1 complex can be reconstituted with the incorporation of Crt into B820 subunits in 1% β-OG. The progress of LH1 formation can be monitored as described above. This approach facilitated the observation of a new short-lived intermediate in the assembly of LH1 termed iB873, which can be converted to the LH1 complex if more Crt is applied [56]. Also this reconstitution method allows the replacement of the native BChl *a* with the modified pigments, such as Ni-BChl *a*, simply by adding its solution (in acetone) into the B820 subunits in 1% β-OG, before the oligomerization of the subunits is induced [55].

5. Summary

The fully reconstituted PCP requires peridinin as the main Crt and apoprotein of N-terminal domain as well as Chl. PCP reconstitution with the modified Chl a, i.e. its analogs, can enhance the light absorption in the NIR region. Although energy transfer from peridinin to Chl a is more efficient compared to that one from peridinin to its analogs, i.e. Chl *b*. Importantly, the energy transfer from peridinin to Chl *a* is close to 100% upon the absorption of light by peridinin. Three reconstitution strategies were developed for reconstituting LH1 in large quantities by use of organic solvent-extracted LH1 components, detergent-dissociated Crt-depleted antenna, and oligomerization of B820 subunit. These reconstitution approaches can manipulate independently its components, especially Crts, for enhancing the light absorption by LH1 complex in the blue-green region which is not covered by BChl, and in the NIR region. Therefore, Crt acts as one of the structural requirements in LH1 complex. Intra-complex energy transfer from Crt to BChl can be varied between 30% to 80%, and depends on the n of Crts incorporated to the reconstituted LH1 complexes. By using these LH1 complexes reconstituted with Crts, antioxidant and photo-protective functions of Crt can be studied. Crts may significantly enhance the stability of photosynthetic complexes against oxidation and their protective effect depends on the type of the oxidant [57]. Photo-protective function of Crt, which quenches the

lowest triplet state of BChl *a* and dissipates the transferred triplet energy as heat, is more efficient in the LH1 rather than in the LH2 complex [58]. The generation of the Crt triplet state increases accordingly with the number of conjugated double bonds of incorporated Crt [52].

Acknowledgements

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