Biochemistry

The inter-monomer interface of the major light-harvesting chlorophyll a/b complexes of photosystem II (LHCII) influences the chlorophyll triplet distribution

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Under strong light conditions, long-lived chlorophyll triplets (3Chls) are formed, which can sensitize singlet oxygen, a species harmful to the photosynthetic apparatus of plants. Plants have developed multiple photoprotective mechanisms to quench 3Chl and scavenge singlet oxygen in order to sustain the photosynthetic activities. The luminal loop of light-harvesting chlorophyll a/b complex of photosystem II (LHCII) plays important roles in regulating the pigment conformation and energy dissipation. In this study, site-directed mutagenesis analysis was applied to investigate triplet–triplet energy transfer and quenching of 3Chl in LHCII. We mutated the amino acid at site 123 located in this region to Gly, Pro, Gln, Thr and Tyr, respectively, and recorded fluorescence excitation spectra, triplet-minus-singlet (Tm5) spectra and kinetics of carotenoid triplet decay for wild type and all the mutants. A red-shift was evident in the Tm5 spectra of the mutants S123T and S123P, and all of the mutants except S123Y showed a decrease in the triplet energy transfer efficiency. We propose, on the basis of the available structural information, that these phenomena are related to the involvement, due to conformational changes in the luminal region, of a long-wavelength lutein (Lut2) involved in quenching 3Chl.

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I n t r o d u c t i o n

Regulation of the structure and function of the major light-harvesting chlorophyll (Chl) a/b complexes of photosystem II (LHCII) has been investigated for decades. Great progress has been made, especially since the discovery of the crystal structure of LHCII at 2.72Å from spinach and at 2.5Å from pea (Liu et al., 2004; Standfuss et al., 2005). The exquisite arrangement of the chromophores, namely 14 Chls and 4 carotenoids (Cars) for each LHCII monomer that comprises three transmembrane α-helices, one amphipathic α-helix and one 310-helix, ensures highly efficient singlet–singlet and triplet–triplet energy transfer, and effective regulation of the structure for avoiding damage from over-excitation under diverse environmental conditions (Frank and Cogdell, 1996). The four Cars in LHCII, namely two luteins (Luts), one neoxanthin (Neo) and one violaxanthin, mainly take part in photoprotection, besides acting as essential components in the LHCII structure and accessory light absorbers. Two Luts (Lut6620 and Lut621) are located in the core of LHCII, forming a cross bridge and embracing the central α-helices A and B. Lut620 has strong interactions with the neighboring cluster Chl610/611/612, which has the lowest site energy (Mühl et al., 2010; Remelli et al., 1999) and can be referred to as terminal emitter locus (Pascal et al., 2005), hence it can easily accept triplet energy from Chl. Lut620 is also located close to Chl613, which forms another cluster with Chl614 in the luminal side. By comparison, Lut621 is in the vicinity of the cluster Chl602/603 in the stromal side and forms a strong excitonic coupling with it. Additionally, Lut621 is located near Chl604, the “bottleneck” of energy transfer in the luminal side (Mühl et al., 2010; Novoderezhkin et al., 2005). Unlike Luts, which are contiguous to Chl, Neo is situated in the Chlb-rich region, interacting with Chlb606 and Chlb608, even in close proximity to Chlb604, Chlb605 and Chlb607, whereas the violaxanthin is in the periphery of LHCII, far from the Chls except Chlb601, in relation to other Cars.

In LHCII, the singlet excitation energy of Cars is transferred with near unit quantum efficiency to Chl. However, under strong light condition, some excess excited Chls, unable to hand over their excitation to the reaction center, undergo intersystem crossing and form Chl triplets (3Chls) which act, on account of their long lifetime, as sensitizers of singlet oxygen (Siefermann-Harms, 1987), a

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harmful reactive species that may damage the photosynthetic apparatus and thus inhibit photosynthesis. Photodamage can be prevented by quenching either any singlet oxygen that is formed or the sensitizing species itself, and Cars are able to carry out both these reactions. The Chl-to-Car triplet–triplet energy transfer is mediated by Dexter mechanism (Dexter, 1953), which needs an overlap between the electron clouds of the donor and acceptor. This implies that strong interactions occur between the adjacent pigments, especially for a Car molecule and its neighboring Chl(s). It has been reported that the rate constant for energy transfer from Chlb to Chla is very much larger than that for the formation of Chlb triplets, and therefore triplets are formed mainly on Chl molecules (Visser et al., 1996). That Lut is better able to quench 3Chl compared to Neo may be inferred from the high resolution structure of LHCII (Liu et al., 2004; Standfuss et al., 2005). Studies elucidating the function of individual Cars show that Lut is involved in the 3Chl quenching whereas Neo is not (Mozzo et al., 2008; Peterman et al., 1997), which is consistent with the above analysis. The experiment on a Lut-deficient mutant also supports the view that Lut is needed for efficient 3Chl quenching in LHCII in vivo (Dall’Osto et al., 2006). However, the hypothesis for the functions of violaxanthin in 3Chl quenching (Mozzo et al., 2008; Peterman et al., 1997) cannot be reconciled with the above view. Moreover, two recent studies have indicated that zeaxanthin plays a role in controlling 3Chl formation in LHCII (Betterle et al., 2010; Dall’Osto et al., 2012).

Despite the increasing interest in the process of triplet–triplet energy transfer and the molecular mechanism of the 3Chl quenching in LHCII, many questions still remain unanswered. Furthermore, scant attention has been paid as to how the conformational change of LHCII affects the triplet energy transfer and quenching. Our previous results indicated that the lumenal loop between helix B and C, which is rather flexible, is important for stabilizing the structure and regulating the function in LHCII (Liu et al., 2008; Yang et al., 2008). Particularly the amino acid residue at site 123 located at the joint of helix C and lumenal loop is of significance in regulating the conformation of LHCII, intermolecular interactions and Chl fluorescence yield (Liu et al., 2008). In this work, in order to find the influence of the conformational changes of lumenal loop on the triplet energy transfer and 3Chl quenching, we mutated Ser123 to Pro(S123P), Gln(S123Q), Thr(S123T) and Tyr(S123Y), together with a previous mutant S123G (Liu et al., 2008), then investigated their fluorescence excitation spectra and triplet-minus-singlet (TmS) spectra. The results showed that the peak at 4Car absorption has an obvious red-shift in the TmS spectra when Ser123 was exchanged to Thr or Pro, which is probably associated with Lut2 involvement in the 3Chl quenching resulting from the conformational changes in the lumenal loop region in LHCII.

Materials and methods

All of the mutants were obtained with the method of site-directed mutagenesis as described previously (Liu et al., 2008). The mutation primers were designed as follows: S123P, 5′-CAAGGATCCTTCGATTCAT-3′; S123Q, 5′-CAAGGATCCTTCGATTCAT-3′; S123T, 5′-CGGTTCATGCTACCAACCA-3′; S123Y, 5′-GCAGAACGATATTTCCAT-3′ (S123G has been used previously (Liu et al., 2008)). The apoprotein of different Lhcb1 species were overexpressed and isolated with the method described by Paulsen et al. (1990). In the presence of isolated total thylakoid pigments, the LHCII was reconstituted according to the method described previously (Liu et al., 2008).

Absorption spectra were recorded at room temperature with UV–1601PC UV–visible spectrophotometer (Shimazu, Japan), which has a spectral band pass of 2 nm. The sampling interval was set to 1 nm with a scanning range from 350 nm to 750 nm. The Chl concentration of each sample was adjusted to 10 μg/ml approximately in a dilution buffer including 12.5% (w/v) sucrose, 20 mM phosphate buffered saline (pH 7.5) and 0.1% (w/v) dodecyl β-D-maltoside (DDM).

Room temperature fluorescence excitation spectra were obtained using a Fluorolog spectrophotofluorometer (Jobin Yvon-SpeX, France), which is equipped with two double monochromators, one for each beam. The spectral band pass for each double monochromator was set to 5 nm, and the sampling interval to 1 nm. Since the excitation wavelength (350–750 nm) was scanned through the emission band (centered at 680 nm), it was necessary to subtract the contribution to the signal made by the scattered light; the profile of the scattered exciting light was recorded by replacing the sample with an aqueous blank. The excitation spectra were corrected by recording the intensity of exciting light in situ, as described previously (Naqvi, 1998), but the Golay detector was replaced by a pyroelectric detector (SciTeck Instruments, UK, model P2613-06).

Transient absorption spectra were measured by using a pulsed laser (EKSPLA, NT342B-SH-10-WW) with pulse energies of 10–15 mJ and of 8 ns duration. The probe light was provided by a pulsed xenon lamp with a duration of 0.5 μs at right angle to the laser pump beam and the signal was detected by an array detector (BWTek, Model BRC642E). The transient absorption spectra were calculated as:

\[
\Delta A(\lambda, t) = \frac{I(\lambda) - I_0(\lambda)}{I_{0}(\lambda, t) - I_{0}(\lambda)}
\]

where \( t \) is the delay time between the laser and the probe flash. \( I(\lambda) \) is the intensity of the transmitted spectrum at wavelength \( \lambda \) measured by the array detector using different light sources: only the probe light (reference or R); no probe light (dark or D); the probe light followed by the laser (sample or S) at a delay \( t \); and only the laser (laser or L). Single-wavelength kinetic traces were recorded by using a commercial instrument (Applied Photophysics, model LSK-160) that uses a pulsed xenon arc lamp as the monitoring source.

Results

The mutagenesis to the residue 123 at the monomeric interface influences the absorption and the fluorescence excitation spectra of LHCII

The shape of the fluorescence excitation spectra of LHCII reflects, when one uses a monitoring wavelength where Chla emission dominates, the efficiency of singlet–singlet energy transfer from the accessory pigments to the emitting species. In order to evaluate the effect of mutagenesis on singlet–singlet energy transfer, we recorded the fluorescence excitation spectra of different LHCII species as well as the corresponding absorption spectra, so that the fluorescence efficiency could be evaluated. Fig. 1 presents the difference spectra of the fluorescence excitation (Fig. 1A) and the absorption (Fig. 1B) of different mutants versus the wild type (WT) LHCII (WT minus mutants). For reference, the fluorescence excitation spectra and the absorption spectra, normalized to the maximum in the Q\(_\beta\) region (ca. 672 nm), presented in the insets of the corresponding difference spectra. The most striking feature of the difference spectra is the parallel variations of the absorption and excitation values at 650 nm and 488 nm, which may be associated with Chlb and Neo, respectively. It is reasonable to suppose that the reduction at 650 nm involves changes in the environment of Chlb. The change at 488 nm absorption may likewise be attributed to a perturbation of Neo, because the 488 nm absorbance corresponds to the 0–0 transition of Neo (Ruban et al., 2001). It stands to reason that, with Neo located in the Chlb-rich region, the two absorbance changes, since they go hand in hand, should be the...
consequence of the Chlb–Neo interaction, which can be evaluated by the height of 488 nm peak. Comparing the Chlb–Neo interaction of different mutants, we see that the interaction is most pronounced in the S123T and S123P mutants. The similarity of the absorption and fluorescence excitation spectra of Fig. 1 indicates that the energy transfer efficiency is not greatly affected by the mutagenesis, which is further verified by the fluorescence efficiency (fluorescence/absorption at 436 nm) calculation (Table 1).

The evaluation of carotenoid and chlorophyll triplets in the different LHCB species

Two excitation wavelengths, 470 nm or 436 nm, were employed to excite mainly Chlb or Chla, and two values of t were used (0.1 and 25 μs), which will be denoted by t1 and t2, respectively. For the sake of convenience, we will introduce the abbreviations $\Delta_1 = \Delta A(\lambda; t_1)$, $\Delta_2 = \Delta A(\lambda; t_2)$, $\Delta_{12} = \Delta_1 - \Delta_2$. Plotting $\Delta_1$, $\Delta_2$ and $\Delta_{12}$ against $\lambda$, we get short (0.1 μs) delay TmS spectra, long (25 μs) delay TmS spectra and their difference spectra, respectively. Fig. 2 shows the TmS spectra of WT LHCII under the condition of excitation wavelength at 436 nm (Fig. 2A) or 470 nm (Fig. 2B). The short delay TmS spectra contains the TmS spectrum of the Cars accepting the triplet energy from the Chls, with a peak at 507 nm, and the TmS spectra of the Chla and Chlb (TmSa and TmSb) negatively peaked at 675 nm and 650 nm, respectively, related to the “disconnected” Chls in the complex which are not able to transfer their triplet energy to the Cars. The lifetime of Chl triplets measured under anaerobic conditions are long, up to several hundreds microseconds, so only the TmS components of Chla and Chlb are present in the long delay spectra, namely, the plots of $\Delta_2$ against $\lambda$ (Fig. 2). The triplet–triplet absorption coefficients of Chla and Chlb are small and change little with wavelength. Accordingly their TmS spectra are dominated by the corresponding ground state depletion signals at the red end. This figure also shows plots of $\Delta_{12}$ against $\lambda$, which are considered as “pure” TmS spectra of the Cars (TmSC), because TmS spectra of disconnected Chls are canceled out by subtracting the long delay TmS spectrum from the short delay one. In spite of this, a small negative dip appears at around 680 nm, which reflects the perturbation of a ground state Chl molecule when its Car neighbor is promoted to the triplet state. Comparing the TmS spectra measured under Chlb and Chla excitations (Fig. 2), it is clear that no remarkable differences could be detected.

The mutagenesis changed the distribution of carotenoid triplets in LHCB

In order to study the influences on the triplet energy transfer resulting from site-directed mutagenesis, the TmS spectra (TmSC, $\Delta_{12}$ against $\lambda$), corrected for the slight differences in absorbance at the laser pump wavelength, of all the mutants were studied (Fig. 3). All of the samples conservatively had a significant positive peak in the Soret region and a small negative peak in the Q2 region. The WT LHCII showed maximal $^3$Car absorption at 507 nm, while the mutant LHCIIIs presented changes in the $^3$Car absorption spectra,
that quenching Gly, triplet et 522. The inset are the enlargements of 3Car absorption peaks in order to show difference in this region among the samples.

Fig. 3. Comparison of the TmS spectra of reconstituted WT LHCII and mutant LHCIs. The spectra were obtained by plotting Δ12 against λ for each sample, and have been corrected for the difference in absorbance at the laser pump wavelength. (A) shows the TmS spectra of WT, S123P and S123T, while (B) shows the TmS spectra of WT, S123G, S123Q and S123Y.

Table 2
The peak position and FWHM of each reconstituted LHCII.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak (nm)</th>
<th>FWHM (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>S123G</td>
</tr>
<tr>
<td>Peak (nm)</td>
<td>507.4</td>
<td>507.9</td>
</tr>
<tr>
<td>FWHM (nm)</td>
<td>23.5 ± 1.2</td>
<td>26.4 ± 0.6</td>
</tr>
</tbody>
</table>

both in the peak height and the shape, to different extents in different mutants. Among all the mutant LHCIs, the S123T or S123P mutants presented the most obvious changes in the peak position, both showed up to 3.5 nm red-shift in the 3Car absorption maxima. Table 2 presented the peak positions and the full width at half maximum (FWHM), calculated based on Fig. 3, of different LHCII species. It is obvious that all the mutagenesis at site 123 widened the major spectral peak of 3Car absorption spectrum, which implied the increased components in the 3Car spectra. It might be possible that the conformational change induced by exchanging Ser123 to Gly, Pro, Gln, Thr or Tyr perturbed the excitonic coupling between Chl and Car, which resulted in changes in the triplet energy distribution in the complexes. According to earlier research (Lampoura et al., 2002), the triplet absorption at 507 nm is ascribed to Lut1 (Lut620, named Lut1 regarding Chl–Car triplet transfer), and at 525 nm belongs to Lut2 (Lut621, named Lut2 regarding Chl–Car triplet transfer), the increased FWHM might possibly be attributed to changes in the Lut2 component that is involved in the 3Chl quenching within LHCII.

To investigate the individual contributions and the lifetimes of Lut1 and Lut2 under normal conditions, the kinetics of triplet absorption over the 490–540 nm range were measured under aerobic conditions, subjected to a singular value decomposition (SVD). The kinetic trace could be well fitted by a biexponential decay, with two spectral components positively peaked at 507 nm and 522 nm respectively (Fig. 4). The component with a maximum at 507 nm (Lut1) had a shorter lifetime and higher amplitude, while the other one (Lut2) possessed longer lifetime and lower amplitude (Table 3). The lifetimes of 3Car absorption decay in this work are shorter than those reported previously (Mozzo et al., 2008; Naqui et al., 1997; Peterman et al., 1995). This should be attributed to the 0.1% DDM concentration we used for the sample because the former research has proposed that the increasing detergent concentration might lower the triplet energy transfer efficiency from Chl to Car (Naqui et al., 1999). Since the purpose of this work is to investigate the influence of site-directed mutagenesis on the triplet energy transfer in LHCII, we focus on comparing the differences between WT and the mutants in the same detergent medium. From Fig. 4, we could see that the yield of triplet Lut1 changed more violently, among different mutants, compared with that of Lut2. The changes in the triplet yield distribution among different mutants resulted in significant change in the ratio of the amplitudes of two components (Lut2/Lut1 = V/22/V/007), among which the mutant S123T had the largest amplitude ratio (Lut2/Lut1 = 0.46) followed by S123P (Lut2/Lut1 = 0.41). This indicated that the mutagenesis, especially in S123T and S123P, influenced the conformation of LHCII, caused redistribution of 3Chl between the two Luts. Comparing the changes of the triplet distribution in Lut2 and Lut1 with changes in of Chlb–Neo interaction strength of different mutants revealed a phenomenon that the mutants which possess stronger Chlb–Neo interaction presented higher triplet distribution to Lut2. The two mutants S123T and S123P with highest Chlb–Neo interaction distributed most triplet energy to Lut2. From this view, it is possible to propose that the changes in the pigment conformation of the Chlb–Neo niche accounted for the changes in the triplet distribution.

Discussion

Carotenoids perform many important roles in the photosynthetic process. Besides acting as essential components in the LHCII structure, they function as accessory pigments in harvesting solar energy, and carry out the vital role of protecting photosynthetic machinery under over-excitation light conditions. One of the photoprotection processes is that Cars deactivate, through triplet–triplet transfer, 3Chl produced by intersystem crossing.

Table 3
The features of spectral components obtained from singular value decomposition under the aerobic condition at excitation wavelength 436 nm.a

<table>
<thead>
<tr>
<th>Samples</th>
<th>V/22/V/007</th>
<th>V/007</th>
<th>V/22 (μs)</th>
<th>V/007 (μs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.32</td>
<td>0.099</td>
<td>1.90</td>
<td>2.74</td>
</tr>
<tr>
<td>S123G</td>
<td>0.28</td>
<td>0.091</td>
<td>2.24</td>
<td>3.24</td>
</tr>
<tr>
<td>S123P</td>
<td>0.41</td>
<td>0.064</td>
<td>2.03</td>
<td>2.95</td>
</tr>
<tr>
<td>S123Q</td>
<td>0.33</td>
<td>0.077</td>
<td>2.17</td>
<td>3.17</td>
</tr>
<tr>
<td>S123T</td>
<td>0.46</td>
<td>0.069</td>
<td>2.13</td>
<td>2.81</td>
</tr>
<tr>
<td>S123Y</td>
<td>0.28</td>
<td>0.090</td>
<td>2.29</td>
<td>3.23</td>
</tr>
</tbody>
</table>

a V/22/V/007 means the ratio of the amplitude of two components (Lut2/Lut1 = V/22/V/007); V/007 the amplitude of Lut1 components; V/22 the lifetime of the component Lut1 and V/007 the lifetime of the component Lut2.
thereby preventing the formation of harmful singlet oxygen, through the quenching of 3Chl by ground-state molecular oxygen. The triplet–triplet energy transfer from 3Chl to Car happens via Dexter electron exchange mechanism (Dexter, 1953) requires the two molecules to be in van der Waals contact. Studies on triplet energy transfer in light-harvesting complex II of purple bacteria showed that the separation between Chl and Car is less than 4 Å for optimal triplet energy transfer (Damjanovic et al., 1999). Based on the crystal structure of LHCII from spinach and pea (Liu et al., 2004; Standfuss et al., 2005), the Chls are situated in the neighborhood of Cars, with two Luts in close proximity to Chla clusters and Neo surrounded by Chlsb. This architecture makes it possible for the effective transfer of the excitation to the reaction center and the quenching of over-excitation to heat under too strong light conditions. Perturbing LHCII complexes using detergents has been used in earlier investigations to show that increasing the detergent concentration lowers the triplet energy transfer efficiency from Chla to Car and raises the yields of 3Chl and fluorescence (Naqvi et al., 1999), which suggests that comparatively small structural alterations can strongly affect the energetic coupling of different pigments and consequently, the distribution of the excitations in the complex. Similarly, in the LHCII mutants, the conformational changes of the complex might lead to changes in the mutual separations and orientations of the pigments as evidenced by the changed absorption and fluorescence excitation spectra (Fig. 1). Thus, the singlet–singlet energy transfer between Chls is perturbed, which in turn, influences the triplet–triplet energy transfer from Chl to Car, as well (Ballottari et al., 2013).

It is widely accepted that Lut contributes to the 3Chl quenching in LHCII (Dall’Osto et al., 2006; Peterman et al., 1995). Our TmS spectra showed a significant peak at around 507 nm, which was regarded, in view of previous reports (Lampoura et al., 2002; Peterman et al., 1995, 1997; Schodel et al., 1998), as the triplet absorbance of Lut1. In addition, a long wavelength 3Car component (525 nm) described by Lampoura et al. (2002), ascribed to the contribution of Lut2, also appeared in our TmS measurement. Especially, the mutants S123P and S123T showed a significant red-shift at the maximum of 3Car absorption, as well as the intensified absorbance was found in the region of 507–550 nm in all of the mutants (see Fig. 3 and Table 2). These indicate that Lut2 is possibly involved, more intensively, in the 3Chl quenching in the mutant LCHCs. In this work, the mutagenesis at site 123 results in the partial loss of Neo, and the changes of pigment conformation, especially in the mutants S123P and S123T (unpublished data). Considering that the circular dichroic signal at 652 nm is strongly dependent on the presence of Neo (Croce et al., 1999), we suppose that the conformational change in the Neo-domain is ascribed to Chlb–Neo interaction. Also the spectral shape of these two mutants compared with WT is similar to that of Neo-deficient reconstituted Lhcb5 reported by Ballottari et al. (2013), which indicates that the changes in TmS spectra perhaps result from the loss of Neo and/or the accompanying conformational disturbance. Fig. 5 plots the Lut2/Lut1 ratio versus the strength of Chlb–Neo interaction, measured as the height of 488 nm band in the difference absorption spectra (see Fig. 1). Obviously S123T and S123P, which both have drastic conformational alteration in the Neo-domain, undergo significant changes of triplet distribution. On the other hand, it has been reported that the triplet absorption at 525 nm increases under the condition of aggregation (Lampoura et al., 2002), and that the 3Car absorption band is red-shifted and broader with a more pronounced interaction band in the Qs region for aggregates (Naqvi et al., 1997).

The structural model provides some clues as to why Lut2 participates in the quenching of 3Chl when there is a conformational change in LHCII. It has been shown that Neo does not directly quench 3Chl, but it affects the process of triplet energy transfer (Mozzo et al., 2008). In this study, we found a close relation between the Chlb–Neo interaction and the red-shift in the TmS spectra, which is strongly influenced from the distribution of triplet to the Lut2. The two mutants (S123T and S123P) which have the most significant changes of Chlb–Neo interaction, also showed the strongest red-shift at the maximum of 3Car absorption in the TmS spectra. From the 3D structure of spinach LHCII (Fig. 6), the closest distance between the conjugated π-systems of Chla604 and Lut2 is merely 4.1 Å, just in excess of the optimum distance for efficient triplet–triplet transfer. Normally, Lut2 contributes little to the quenching of 3Chl; however, it becomes effective when the distance between the two Luts is approximately 3.9 Å (Kulmala et al., 2000).

Fig. 4. The TmS spectral components of WT LHCII and mutants obtained by singular value decomposition of the decay spectra, measured over the range 400–540 nm, excited at 436 nm under aerobic conditions. (A) shows two spectral components of WT, S123P and S123T, while (B) shows those of S123G, S123Q and S123Y. c1 and c2 represent the 507 nm component and the 522 nm component, respectively.

Fig. 5. The relationship between the ratio of two Lut components (Lut2/Lut1) and the degree of Chlb–Neo interaction for WT and mutant LHCII. The degree of Chlb–Neo interaction was measured as the height of 488 nm peak in the difference absorption spectra.


Fig. 6. A plausible structural model of Neo-domain regulating the triplet energy quenching within LHCCI based on the crystal structure of Liu et al. (2004). The protein structure: part of α-helices B/C and the luminal loop are gray. The related Chls (only shown with their porphyrin) and Cars, according to the nomenclature of Liu et al. (2004). The mutation site (Ser123) is presented in magenta. The four arrows denote the conformation of Luts switched from an “unquenched state” to a “quenched state” (Wentworth et al., 2003).

3Chl quenching due to its larger separation. In contrast, Chl610 and Chl612 embrace Lut1 with a distance of about 3.6 Å, and form strong excitonic coupling with it (Liu et al., 2004). Pascal et al. (2005) proposed that Lut1 together with Chl610/a611/a612 cluster may be considered as a potential quenching center in view of Car as an acceptor of excess excitation (van Amoneron and van Grondelle, 2001). Since the Chl cluster a610/a611/a612, which is shown to have the lowest site energy (Georgakopoulou et al., 2007; Müh et al., 2010; Novoderezhkin et al., 2005; Remelli et al., 1999), is thought as the terminal emitter locus in LHCCI, transfer of the triplet excitation energy to Lut1 is more likely. In addition, the epoxycyclohexane ring of Neo hangs over the porphyrin of Chl604, and hydrogen bonds to the phenolic hydroxyl group of Tyr112 (Liu et al., 2004). When Neo is removed from this domain, the hydroxyl group of Tyr will reorient from “blue” to “red” conformation, corresponding to a 10 nm red-shift in the WT-minus-mutant difference spectrum, which is related to Chl604 (Müh et al., 2010). Moreover, Chl604 is the “bottleneck” of excitation energy distribution in LHCCI, and it has quite a slow energy transfer constant (Müh et al., 2010; Novoderezhkin et al., 2005). This implies that it may play some important role in the energy transfer from Chl to Car. The mutagenesis, in our experiment, affects the Neo binding or conformation (see Fig. 6), so the conformational changes of this domain possibly make Chl604 approach Lut2 in a greater degree especially in the mutant S123P and S123T, thus it meets the Dexter contact. This will lead to Lut2 being involved in the 3Chl quenching.

The mutagenesis at Ser123 altered the distance between Chls and Cars as discussed above, resulting in different triplet transfer efficiencies in the mutants. Interestingly, the mutants revealing the largest Chlb–Neo interaction presented the most pronounced red-shift of the 3Luts TmS spectrum, the smallest Lut triplet yield and highest Lut2/Lut1 ratio, indicating altered triplet distribution in the complexes. It seems that Neo plays important roles in the triplet distribution in LHCCI, because loss of Neo affinity (increased ChlB–Neo interaction) to the LHCCI resulted in altered triplet energy distribution. It has been proposed that the conformation of LHCCI is able to switch between the unquenched state and quenched state depending on the light conditions (Wentworth et al., 2003). Under normal conditions, Lut1 is more involved in the 3Chl quenching, while Lut2 can be switched to quenched state by subtle changes in the monomeric interface characteristics at the luminal site (see Fig. 6). Perhaps substituting Ser123 to Pro or Thr changes the conformation of Lut2 to “quenched state”, which results in more triplet distribution to Lut2.

In conclusion, the junction of helix C and the luminal loop in the monomeric interface of LHCCI take part in regulating the triplet distribution and energy transfer efficiency. The mutagenesis of specific amino acid in this region results in changes of Neo conformation that perturb the distance between the related Chls and Cars, and accordingly the degree of pigment interactions, and redistribution of triplet energy.

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References


Lampoura SS, Barzda V, Owen GM, Hoff AJ, van Amoneron H. Aggregation of LHCCI leads to a redistribution of the triplets over the central xanthophylls in LHCCI. Biochemistry 2002;41:9139–44.


