PHOTOSYNTHETICA 44 (4): 615-621, 2006

Effect of detergent on aggregation of the light-harvesting chlorophyll *a*/*b* complex of photosystem 2 and its impact for carotenoid function and fluorescence quenching

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Abstract

Spectroscopy was used to investigate the fluorescence quenching mechanism in light-harvesting complex 2 (LHC2). The 77 K fluorescence excitation spectroscopy was performed for detection of aggregation state of LHC2 treated with different concentrations of octylphenol poly(ethyleneglycol ether)$_{10}$ (TX-100). Resonance Raman (RR) spectra excited with 488, 496, and 514 nm provided molecular configuration of neoxanthin, lutein 1, and lutein 2, respectively. At increased concentration of TX-100, the RR signals of xanthophylls were enhanced in the four frequency regions, which was accompanied with increase of fluorescence of chlorophyll (Chl) *a*. Thus the absorption of the three xanthophyll molecules was inclined to excitation wavelength, which proved that functional configurations of xanthophyll molecules in LHC2 were vital for fast transfer of excitation energy to Chl *a* molecules. Changes in the ν4 region (C-H out-of-plane bending modes, at ~960 cm^{-1} in RR spectra) demonstrated that the twist feature of neoxanthin, lutein 1, and lutein 2 molecules existed in LHC2 trimers, however, it was lost in the LHC2 macro-aggregates. In the second derivative absorption spectra of LHC2, neoxanthin absorption was not detected in LHC2 macro-aggregates, while evident absorption was found in LHC2 trimers and this absorption decreased obviously when TX-100 concentration was higher than 1 mM. Hence the neoxanthin molecule had a structural role in formation of LHC2 trimers. The RR and absorption spectra also implied that carotenoid molecules constructed the functional LHC2 trimers *via* their intrinsic configuration features, which enabled energy transfer to Chl *a* efficiently and led to lower fluorescence quenching efficiency. In contrast, these intrinsic twist configurations were lost in LHC2 macro-aggregates and led to lower energy transfer efficiency and higher fluorescence quenching efficiency.

Additional key words: configurative transformation; energy transfer; Raman spectra; TX-100; xanthophylls.

Introduction

The plant major light-harvesting complex (LHC2b) consists of three nuclear-coded polypeptides, namely Lhcb1, Lhcb2, and Lhcb3 (Jansson 1994). It accounts for about 30 % of the total protein in plant chloroplast membranes and is the most abundant membrane protein. Also it combines about 50 % of total chlorophylls (Chls) in chloroplast (Peter and Thornber 1991). LHC2 has four important roles (Standfuss *et al*. 2005): collects sunlight and transfers excitation energy to photosystem (PS) 2 reaction centre (RC) where a charge separation occurs, distributing excitation energy between PS2 and PS1 through its phosphorylation, maintaining the tight oppression of thylakoid membranes in chloroplast grana, and protecting photosystems from damage by excessive sunlight.

The functional unit of LHC2 is a trimer, which contains usually three monomers and consists of 8 Chl *a* and 6 Chl *b*, 4 Cars (2 luteins, 1 neoxanthin, and 1 violaxanthin), and 2 lipids (phosphatidylglycerol and digalactosyldiglycerol) (Liu *et al.* 2004b, Standfuss *et al.* 2005). The LHC2 trimer has a strong tendency for aggregation *in vitro* or *in vivo*; this results in fluorescence

Received 14 February 2006, *accepted* 3 April 2006.
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Abbreviations: Car – carotenoid; Chl – chlorophyll; LHC2 – light-harvesting chlorophyll *a*/*b* complex of photosystem 2; PS – photosystem; RC – reaction centre; RR – Resonance Raman; TX-100 – octylphenol poly(ethyleneglycol ether)$_{100}$.
Acknowledgements: We thank Prof. Yulong Liu (Institute of Physics, Chinese Academy of Sciences) for his help in the Resonance Raman spectroscopy experiments. This work was supported by the National Sciences Foundation of China (grant No. 30500101 and 30470149).

quenching (Barzda *et al.* 1996, Kirchhoff *et al.* 2003). Aggregation of LHC2 brings an increase of the fluorescence quenching efficiency, which prevents damage to the RC from excess photons. In addition, changes in the association state of LHC2 are thought to be part of the regulatory mechanisms that optimize light-harvesting function under different irradiances (Robert *et al.* 2004). Dynamic balance of the LHC2 trimer and macro-aggregation not only regulate photon absorption and transfer, but also play the role in escaping from photodamage. Many papers try to clarify fluorescence quenching mechanism by which aggregation of LHC2 induces energy dissipation. Cars in LHC2 may play an important role in this mechanism (Ruban *et al.* 2000, 2001, Wentworth *et al.* 2003, Standfuss *et al.* 2005). LHC2 binds five types of xanthophylls: lutein, neoxanthin, violaxanthin, zeaxanthin, and antheraxanthin. The last three are involved in the xanthophyll cycle (Müller *et al.* 2001). These Cars have different affinity to LHC2, neoxanthin and lutein have very strong binding affinities to LHC2 compared with violaxanthin, which can be easily removed by detergent (Ruban *et al.* 1999, 2001). The two luteins of LHC2 monomer are located near the trans-membrane helixes A and B in the inner core of LHC2 and probably have a structural role (Ruban *et al.* 2001, Liu *et al.* 2004b). Their polyene chains are inclined with respect to the membrane normal by angles of about 59^o and 62^o, respectively (Liu *et al.* 2004b). Neoxanthin (9-cis) is located in the Chl *b*-rich region around helix C. Its polyene chain forms an angle of about 58^o with the membrane normal (Ruban *et al.* 2001, Liu *et al.* 2004b).

Addition of detergent decreases lateral aggregation of LHC2, but not the stacking (Kirchhoff *et al.* 2003). Bassi and Caffarri (2000) assumed that the lateral aggregation of LHC2 trimers enables new Chl interactions at the periphery of the complexes, which could serve as a trap for excitation energy. Wentworth *et al*. (2003) presented a putative model in which the configurative changes of lutein molecules (lutein 1 domain) respond to fluorescence quenching efficiency in LHC2. According to Standfuss *et al.* (2005) the 0.25 nm resolution structure proposed a mechanism for xanthophyll-related photo-protection in LHC2. They considered that there are two close Chl *a*-lutein pairs (Chl 2/lutein 1 and Chl 5/lutein 2 pairs) in every LHC2 monomer; this interaction would cause a red-shift in the absorption of Chl 2 and Chl 5. This red-shift converts Chl 2 and Chl 5 into sinks for Chl triplets, and then Chl triplets are defused by the luteins. So luteins may play important roles in photo-protection (Standfuss *et al.* 2005). However, the quenching mechanism is still not clear. The direct proof of dynamic alteration in the configurative conversion of Cars in LHC2 is still missing. Also, why the LHC2 macro-aggregates have more efficient fluorescence quenching rate than is that of LHC2 trimers and what would happen at molecular level of Cars during the state conversion of LHC2 trimers and macro-aggregates? Precise answers are still absent and more studies of construction and functions of LHC2 are necessary.

The critical micelles concentration of octylphenol poly(ethyleneglycol ether)$_{100}$ (TX-100) is around 0.31 mM (Montoya *et al.* 1994). TX-100 is able to form micelles with the membrane lipids and binds predominantly to hydrophobic protein domains (Kirchhoff *et al.* 2003). As a result, the native lipid bilayer is perturbed and the membrane proteins are partially de-lipidated (Popot and Engelman 1990, Lau and Bowie 1997). Ruan *et al.* (2000) showed that short-time treatment (15 min) with TX-100 perturbed the structure of membrane lipids, but had no clear effect on the protein secondary structure. The presence of detergents could change the state of aggregation of LHC2 trimers and the concentration of detergent was close related to the Chl *a* fluorescence quenching efficiency in LHC2 (Ide *et al.* 1987, Ruban *et al.* 1997, Kirchhoff *et al.* 2003). Kirchhoff *et al*. (2003) indicated that 500 μM TX-100 is the ideal concentration for LHC2 trimer formation. Based on the above facts, we employed the final concentrations of TX-100 of 0, 0.1, 0.3, 0.5, 1.0, 1.5, and 2.0 mM in LHC2 preparations (macro-aggregates) to produce the kinetic course of conversion in different states of LHC2.

As the dynamic transformation among different LHC2 states and the more direct proof for the above mechanism are absent, the mechanism of fluorescence quenching in LHC2 is still dubious. And the exact site of energy dissipation within the antenna remains uncertain (Wentworth *et al.* 2004). We used various spectral methods, such as Resonance Raman (RR) spectra, fluorescence excitation and absorption spectra, to study the dynamic changes of pigment molecule configurations during LHC2 state conversion. The aim of our work was to further explain the function of molecular configuration of Cars in fluorescence quenching mechanism in LHC2.

Materials and methods

Preparation of LHC2 was done according to Krupa *et al.* (1987) with some modifications. PS2 membrane pellet (Berthold *et al.* 1981) was washed twice with 5 mM EDTA, 50 mM sorbitol, and 50 mM Tricine-NaOH buffer (pH 7.8), and centrifuged at 10 000×*g* for 10 min. The pellet was re-suspended with cold distilled H_2O to a Chl concentration of 0.8 kg m^{-3} and then *Triton X-100* was added from 5 % (m/v) stock solution to a final detergent concentration of 0.7 % (m/v). The suspension was incubated at room temperature with continuous stirring for 30 min and centrifuged at 30 000×*g* for 40 min. KCl and $MgCl_2$ from 1 M stock solutions were added to *Triton X-100* supernatant to final concentrations of 100 and 20 mM, respectively, and stirred gently for the

precipitation of crude LHC2. The suspension was then layered on a 0.5 M sucrose solution (the volume of sucrose should exceed three times the volume of LHC2 suspension) and centrifuged at 10 000×*g* for 10 min. The pellet was re-suspended in 50 mM Tricine-NaOH buffer (pH 7.8) containing 100 mM sorbitol to a Chl concentration about 0.8 kg m^{-3}. *Triton X-100* (5 % m/v stock solution) was then added to obtain a detergent to Chl ratio of 10 : 1. After short stirring, LHC2 was precipitated with K^+ and Mg^{2+} salts as described above. The suspension was gently stirred to allow good precipitation of LHC2 and then it was layered on 0.5 M sucrose solution and centrifuged at 30 000×*g* for 40 min. For the final purification of LHC2, the pellet was suspended in 50 mM Tricine-NaOH buffer (pH 7.8) containing 100 mM sorbitol and precipitated with 1 M KCl and 1 M $MgCl_2$ as described above (Krupa *et al.* 1987). *Triton X-100* was omitted in this step of purification. The suspension containing LHC2 was layered on 0.5 M sucrose solution, centrifuged at 10 000×*g* for 10 min, and finally, the purified LHC2 (macro-aggregates) was suspended in the 50 mM Tricine-NaOH buffer (pH 7.8), frozen in liquid nitrogen, and stored at –80 °C. LHC2 monomers were prepared by phospholipase A_2 treatment and sucrose density gradient centrifugation as described in Ruban *et al.* (1999).

The TX-100 treatment was done by addition of TX-100 to LHC2 macro-aggregates, and TX-100 final concentrations of 0, 0.1, 0.3, 0.5, 1.0, 1.5, and 2.0 mM were reached, respectively. Also, the LHC2 buffer was complemented for achievement of equal final Chl concentrations. Spectroscopy was carried out after 6-min treatment with different concentrations of TX-100 and at the same Chl concentrations according to requirements of fluorescence spectra, RR spectra, and absorption spectra measurements, respectively.

Spectroscopy: Steady state 77 K fluorescence emission spectra were recorded with a fluorescence spectrometer (*F-4500*, *Hitachi*, Japan) at a Chl concentration of 10 g m^{-3}. The optical band width was 4 nm and excitation wavelength was 480 nm where Chl *a* absorption is negligible.

RR spectra were obtained using a Raman spectrophotometer (*JY-T6400*, *Jobin-Yvon*, France). Excitation was provided with an argon ion laser (*SP-165-09*, *Spectra Physics*, USA) at 488, 496, and 514 nm. The measurement was processed under microscopy equipment and Chl concentration was at 60 g m^{-3}. The resolution of the RR spectrometer was 2 cm^{-1}.

The room temperature absorption and the second derivative absorption spectra of LHC2 samples were obtained using a spectrophotometer (*DU-800*, *Beckman*, USA) at a Chl concentration of 10 g m^{-3}. The wavelength interval was 0.2 nm and the scan speed 240 nm per min.

Chl pigments were extracted with 80 % acetone and their concentration was determined according to Arnon (1949) using the *DU-800* spectrophotometer.

Results

77 K fluorescence emission: The ratio of F_{700} to F_{680} can be used to evaluate the level of LHC2 aggregates and F700 representing fluorescence quenching of Chl *a* (Ruban *et al.* 1997, Kirchhoff *et al.* 2003). As shown in Fig. 1, the peak of F700 was remarkable in LHC2 macro-aggregates (without TX-100), which indicated that LHC2 became aggregated. In other samples containing TX-100, the value of F_{700}/F_{680} decreased abruptly and the peak of F_{700} was lost, which implied that LHC2 was trimerized. The samples treated with 1.5 and 2.0 mM TX-100 showed a peak at 650 nm representing free pigments (Kirchhoff *et al.* 2003) and suggesting that the pigment-protein complex is disrupted in these two samples and the two TX-100 concentrations are too high for a functional LHC2 trimer. The decrease of TX-100 concentration induced the red shift of position of the maximum fluorescence emission, indicating a modification of the micro-environment around the Chl *a* molecules upon treatment with different TX-100 concentrations.

RR excitation spectroscopy: RR spectra of Car molecules have four main frequency regions assigned as follows (Ruban *et al.* 2001, Liu *et al.* 2004a): ν1 (1 350~1 540 cm^{-1}), C=C stretching vibration, it is

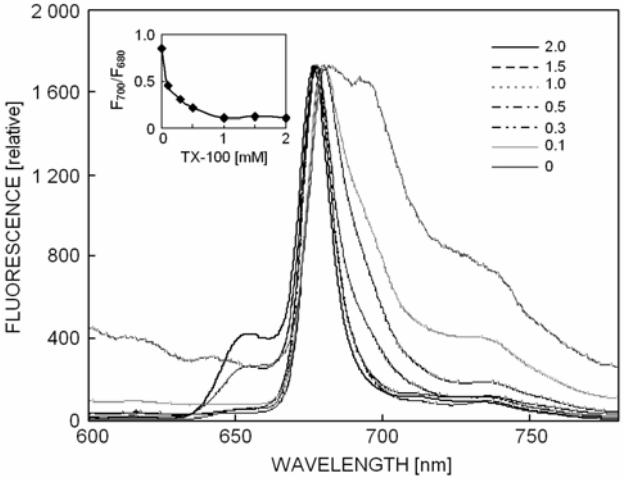

Fig. 1. 77 K fluorescence emission spectra of LHC2 samples after the addition of TX-100 at the final concentrations of 0, 0.1, 0.3, 0.5, 1.0, 1.5, and 2.0 mM. The samples were excited at 480 nm when Chl *a* absorption is negligible, and spectra were normalized to the maximum fluorescence emission value. *Insert*: Changes of F_{700}/F_{680} of LHC2 treated with different concentrations of TX-100.

sensitive to the molecular conformation (trans and cis) of molecule; ν2 (1 100~1 400 cm^{-1}), C-C stretches coupled either to C-H in-plane bending or C-CH3 stretching; ν3 (~1 000 cm^{-1}), CH3 in-plane rocking vibrations; ν4 (at ~960 cm^{-1}), C-H out-of-plane bending modes, this region was very low in intensity. Its intensity depended on the molecular configuration of Cars (distortions from the planar) and the configuration was altered by environment changes such as protein environment, polar environment, *etc*.

The 0-0 transitions of neoxanthin and lutein 1 were located at 486 and 495 nm, respectively. Lutein 2 is at 510 nm in trimeric LHC2 (Peterman *et al.* 1997, Gruszecki *et al.* 1999, Ruban *et al.* 2000, 2001). In our experiment, laser lines at 488, 496, and 514 nm were employed to excite selectively Car molecules in LHC2. The RR spectra were excited at 488, 496, and 514 nm which favoured neoxanthin, lutein 1, and lutein 2, respectively.

In Fig. 2*A*, the strength of the four main frequency regions under different excitation wavelengths (488, 496, 514 nm, respectively) was enhanced. This might imply that the absorption of Cars changed and inclined to excitation wavelength (488, 496, and 514 nm, respectively) when LHC2 was treated with different TX-100 concentrations. So the combination state of the Cars with protein, namely its protein environment, altered the different aggregation state of LHC2. In addition, there was a background of fluorescence intensity of Chl *a* in RR measurements, the fluorescence intensity being shown as a baseline in the RR spectra. The fluorescence of Chl *a* increased gradually with addition of TX-100 (Fig. 2*A*). The RR spectra showed a close relation between the configuration of Cars and the fluorescence of Chl *a*.

The ν4 region corresponds to the transformation when Car molecules are distorted from their planar configuration (Ruban *et al.* 2002, Liu *et al.* 2004a, Andreeva and Velitchkova 2005). In the ν4 region in three laser lines the excitation displayed significant changes and was relatively strong at around 960 cm^{-1} in samples treated

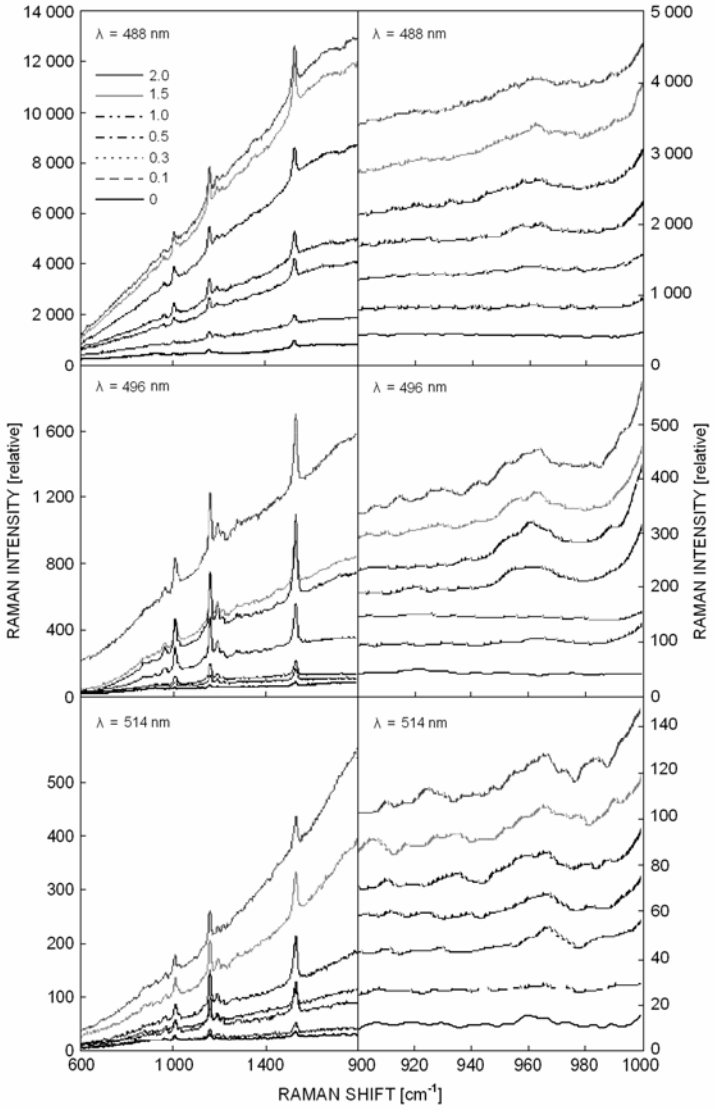

Fig. 2. (*A*) Resonance Raman spectra of LHC2 upon 488, 496, and 514 nm excitation at a chlorophyll (Chl) concentration of 60 g(Chl) m^{-3} after addition of TX-100 at the final concentrations of 0, 0.1, 0.3, 0.5, 1.0, 1.5, and 2.0 mM. (*B*) Resonance Raman spectra in the ν4 region upon 488, 496, and 514 nm excitation at a Chl concentration of 60 g(Chl) m^{-3}. The TX-100 concentrations corresponded to 0, 0.1, 0.3, 0.5, 1.0, 1.5, and 2.0 mM. Spectra were shifted on the y-axis for better clarity

with more than 0.3-mM TX-100 (Fig. 2*B*). This indicated that the twist configuration of Cars (neoxanthin, lutein 1, and lutein 2) in LHC2 emerged when TX-100 concentration was above 0.3 mM and had been strengthened during trimerization. This observation was consistent with the results of Ruban *et al*. (2000) indicating that lutein molecule was distorted in the LHC2 trimer. On the other hand, these Car molecules lost their distortion configuration in LHC2 macro-aggregates, which was different from the study of Ruban suggesting that oligomerisation of LHC2 trimers led to a specific distortion of the neoxanthin molecule (Ruban *et al.* 2000). These results implied that the twist configuration of Cars is necessary for the functional construction of LHC2 trimers.

Room temperature absorption spectroscopy: The Q_y region of LHC2 (Fig. 3*A*) showed two bands around 650 and 675 nm, which were attributed to the absorption of Chl *b* and Chl *a*, respectively (Croce *et al.* 1999). In the second derivative spectra shown in Fig. 3*B*, the absorption of Chl *a* at 675 nm was separated into two Chl *a* species with different combination state, one absorbing at 685 nm and the other at 673 nm. The peak at 685 nm in macro-aggregates was blue-shifted gradually with the increase of TX-100 concentrations and finally shifted to 673 nm in the sample treated with 2 mM TX-100. In Soret region of LHC2 absorption spectra shown in Fig. 3*A*, 438 nm was attributed to Chl *a* and 470 nm to Chl *b* and Cars. The latter peak blue-shifted by 8 nm from 471 nm treated with 0.1 mM TX-100 to 463 nm treated with 2.0 mM TX-100. This implied that the Car absorption does not disappear with the rise of TX-100 concentration but the bands are simply shifted towards shorter wavelengths. Also, this peak domain was detached to two peaks at 476 and 486 nm in macro-aggregates (free TX-100), which indicated that this peak domain arose from Chl *b* and Cars (mainly neoxanthin), respectively. The data of Ruban *et al.* (2001) suggest that the 485 and 695 nm bands in the second derivative spectrum arise from neoxanthin and lutein 1 molecules in the LHC2 monomer, respectively. According to our data, the peak at 487 nm observed in the second derivative spectra (Fig. 3*B*) might come from neoxanthin molecules (Ruban *et al.* 2000, 2001). The intensity of peak at 487 nm was higher and possessed larger absorption in samples treated with 0.1, 0.3, 0.5, and 1.0 mM, however, it decreased at 1.0 mM and higher TX-100 concentrations and was very weak in macro-aggregates (without TX-100).

Fig. 3*C* shows that the absorption at 495 nm corresponding to the absorption of lutein 1 (Wentworth *et al*. 2004) decreased with the increase of TX-100 concentration, which lead to an elevation in peak value of 495 nm in the absorption difference spectra. This decrease in absorption indicates that energy absorbed by lutein 1 molecules decreased, which may arise from the change of configuration of lutein 1 molecules when treated with

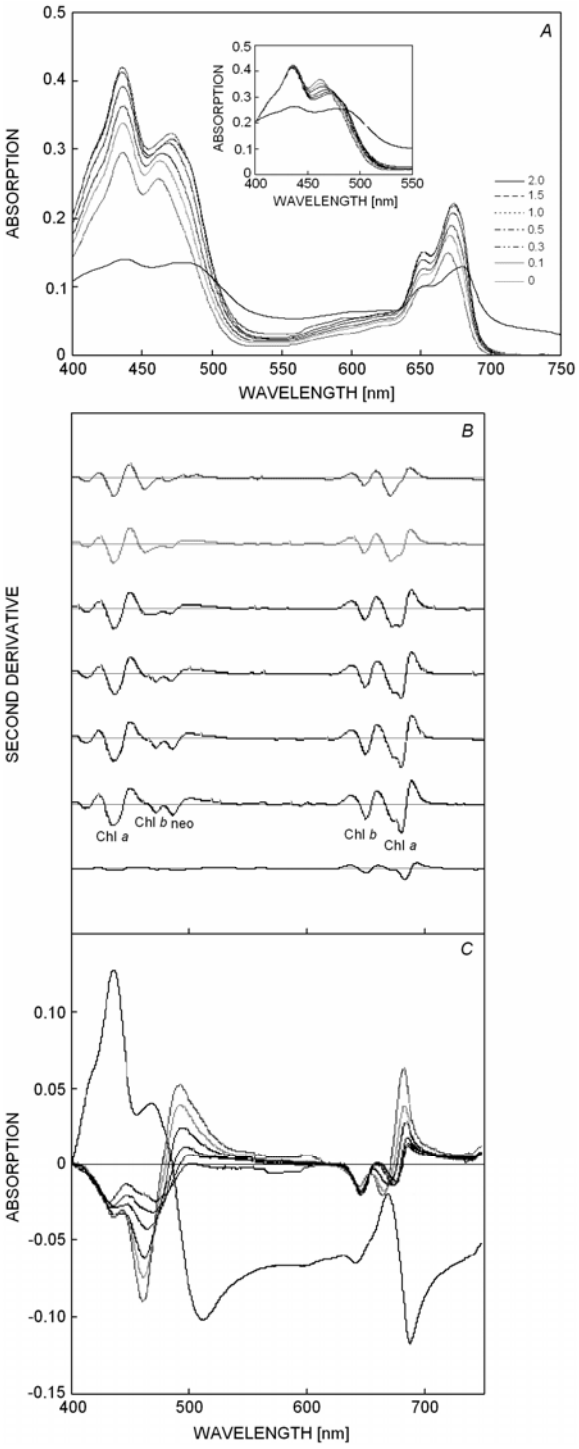

Fig. 3. (*A*) Room temperature absorption of LHC2 treated with different final concentration of TX-100 as 0, 0.1, 0.3, 0.5, 1.0, 1.5, and 2.0 mM. *Insert*: The Soret region of room temperature absorption spectra of LHC2. Spectra were normalized at 400 nm. (*B*) The second derivative absorption spectra of LHC2 samples after the addition of TX-100 at the same final concentrations of TX-100. (*C*) The absorption difference spectra of monomer *minus* LHC2 samples after the addition of TX-100 at the same final concentrations. Corresponding curves in *B* and *C* (500 nm) are from bottom to top.

TX-100. Especially, higher absorption of lutein 1 (much lower peak value at 495 nm in absorption difference spectra) in the LHC2 macro-aggregates was observed than that in LHC2 trimers; this indicated that more energy were absorbed by lutein 1 molecules in macro-aggregates.

Discussion

Cars consist of long, polyenic chains and play important roles in photosynthesis. Among biological molecules, they exhibit the highest resonance Raman cross-section. RR excitation spectroscopy is a powerful approach, which can provide precise information on the conformation and configuration of Car molecules in photosystem (Ruban *et al.* 2001).

RR spectroscopy of LHC2 was performed firstly by Ruban *et al*. (1995) who investigated the changes occurring upon LHC2 aggregation. But further studies were necessary for clarification of configurative transformation of LHC2 pigments, especially Car molecules. Since the binding of Cars to the proteins affected mainly the ν4 region without changing the ν1 region (Ruban *et al.* 2000, 2001, 2002), transitions in the ν4 region were allowed only when Car molecules were distorted from their planar configuration. In our study, the RR spectra excited at 488, 496, and 514 nm were measured for configuration analysis of Car molecules in LHC2 samples treated with TX-100. In RR spectra of the ν4 region (960 cm^{-1}), the configuration of lutein 1, lutein 2, and neoxanthin changed from the non-twist state to twist state when LHC2 macro-aggregates became trimerized. This observation confirmed that the configurative transformation of Car molecules truly exists and plays significant modulating role in varied aggregation states of LHC2. It implies that the configurations of all the three Car molecules were involved in formation of LHC2 trimers and contributed to fluorescence quenching mechanism of Chl *a*. The re-array of their orientation in trimeric LHC2 enabled the successful excitation energy flow to Chl *a*, which led to high fluorescence yield. In contrast, the three Car molecules lost their twist characterization and became Chl *a* quenchers in LHC2 macro-aggregates. Wentworth *et al.* (2004) suggested that the lutein 1 domain was the site of fluorescence quenching and the lutein 2 domain controlled the configuration of the lutein 1 domain, thereby providing allosteric control of fluorescence quenching in LHC2. The crystal structure of spinach LHC2 at 0.272 nm resolution shows that neoxanthin, lutein 1, and lutein 2 molecules interact with the protein helices through Van der Waals contacts and hydrogen bonds (Liu *et al.* 2004a). Based on our experimental results and previous related reports, we suggest that the three Car molecules construct the functional LHC2 trimers *via* their intrinsic configurations and their twist configuration of polyenic chains is important for stabilization of the three-dimensional structure of LHC2. When environments of protein or lipid are destroyed by environmental factors, such as excessive photons or detergents, these intrinsic twist configurations are lost, which leads to lower energy transfer efficiency and higher quenching efficiency occurring between Chl and Car molecules.

Cars function not only in capturing photons but also in transfer of their energy. Two lutein molecules in the LHC2 monomer may have a structural role (Kühlbrandt *et al.* 1994, Ruban *et al.* 2001, Wentworth *et al.* 2003, Standfuss *et al.* 2005). Their contribution to photon capturing in LHC2 is comparatively less than that of Chls (Standfuss *et al.* 2005). There is a difference in lutein orientation in quenched LHC2 aggregates compared with unquenched trimers (Ruban *et al.* 1997, Wentworth *et al.* 2003). In our experiments (Fig. 3*C*), the lutein 1 molecules in LHC2 macro-aggregates absorbed more energy based on the character of their configuration and dissipated the majority of energy, which resulted in lesser energy transfer to Chl *a* from Chl *b*. So LHC2 macro-aggregates displayed lesser Chl *a* fluorescence and showed higher fluorescence quenching efficiency. However, the configuration of lutein 1 molecules changed in trimers as shown in RR spectra, which enabled it to absorb less energy, and the excitation energy from Chl *b* was transferred primarily to Chl *a*. So Chl *a* received more energy and was emitting more fluorescence, having lower fluorescence quenching efficiency. So we propose that fluorescence quenching mainly arises from the contribution of lutein 1; this may be explained by the near central location of lutein 1 in the LHC2 monomer (Kühlbrandt *et al.* 1994, Standfuss *et al.* 2005).

The RR spectra showed that the molecular configuration of neoxanthin changed during trimerization of macro-aggregates. In the second derivative absorption spectra, the peak intensity at 486 nm contributed by neoxanthin molecule (Ruban *et al.* 2000, 2001) was stronger in samples treated with 0.1, 0.3, 0.5, and 1.0 mM TX-100 than in samples treated with 1.5 and 2.0 mM TX-100. However, it was much weaker and not detected in macro-aggregates. As the neoxanthin molecule is located in the external surface and protrudes into the lipid bilayer (Standfuss *et al.* 2005), we conclude that the orderly configuration of neoxanthin molecule existing in trimers was lost in macro-aggregates (without TX-100). On the other hand, lipid or protein environments of neoxanthin can be destroyed by TX-100 concentrations above 1 mM, which comes from the disturbed function of TX-100 to membrane lipid bilayer (Ruan *et al.* 2000). This also demonstrated that the 1 mM TX-100 did a lot of damage to the structure of LHC2. We suggest that neoxanthin molecule stabilizes the construction of LHC2 trimer and plays a structural role in the LHC2 trimer.

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