Investigation of detergent effects on the solution structure of spinach Light Harvesting Complex II

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Abstract. The properties of spinach light harvesting complex II (LHC II), stabilized in the detergents Triton X-100 (TX100) and n-Octyl-β-D-Glucoside (BOG), were investigated by small-angle neutron scattering (SANS). The LHC II-BOG scattering curve overlaid well with the theoretical scattering curve generated from the crystal structure of LHC II indicating that the protein preparation was in its native functional state. On the other hand, the simulated LHC II curve deviated significantly from the LHC II-TX100 experimental data. Analysis by circular dichroism spectroscopy supported the SANS analysis and showed that LHC II-TX100 is inactivated. This investigation has implications for extracting and stabilizing photosynthetic membrane proteins for the development of biohybrid photoconversion devices.

1. Introduction
Light harvesting complex II (LHC II) carries out the first step in photosynthesis by directing photons to the reaction centers of the photosynthetic apparatus of higher plants and algae. It also plays a structural role by maintaining the supramolecular organization in the thylakoid membranes and is involved in the regulation of photosynthesis by dissipating excess excitation energy and controlling the activity of Photosystems I and II. The structures of spinach and pea LHC II have been resolved by X-ray crystallography at a resolution of 2.5 Å and 2.72 Å, respectively. Each subunit of the monomeric polypeptide chain is composed of ~232 amino acids with 3 membrane spanning helices that bind and orient 14 Chlorophylls (Chl) and 4 carotenoids. These studies have identified the position and orientation of the 8 Chl a, 6 Chl b and 4 carotenoids per monomer.

Functional LHC II trimers are typically isolated and purified by partial solubilization of thylakoid membranes using mild non-ionic detergents such as Triton X-100 (TX100), n-Octyl-β-D-glucoside (BOG), and n-dodecyl-β-D-maltoside. The properties of purified LHC II stabilized in detergent solution have been investigated using techniques such as fluorescence and circular dichroism (CD) spectroscopies. These studies provide excellent characterization of the spectroscopic properties of the detergent-associated LHC II but do not provide direct structural information about this system.

The development of photoconversion devices based on natural photosynthetic proteins, such as LHC II, requires the purification of structurally intact and functional membrane proteins and complexes. Protein-detergent complexes are ideally suited for study by SANS with contrast variation because proteins and detergents have inherently different scattering length densities, making contrast variation possible without isotopic labeling of the sample. In this report, we present a comparison of...
the effects of TX100 and BOG on the structure of LHC II isolated from spinach leaves using circular dichroism spectroscopy and small-angle neutron scattering (SANS).

2. Materials and Methods

2.1. LHC II purification

LHC II was purified from market spinach leaves using two different approaches. The method for isolation of the thylakoids and their solubilization in 0.8% (w/v) TX100 was the same in both procedures. For the LHC II-BOG preparation, the solubilized membranes were fractionated by density gradient ultracentrifugation over a continuous 0.1 to 1.0 M sucrose gradient followed by separation of free Chl from LHC II by Mg$^{2+}$/K$^+$ cation-induced appression. The LHC II containing pellet was dissolved in 10 mM Tris HCl pH 7.6 containing 1.8% BOG, 15.5 or 100 % D$_2$O, at a protein concentration of 3.95 mg/ml. The LHC II-TX100 preparation was purified as described earlier.

The sample for SANS analysis was dissolved in 10 mM Tris HCl pH 7.6 containing 1.7 % TX100, in 25% or 100 % D$_2$O, and 0.93 mg protein /ml. CD spectra were recorded using a Jasco 810 CD spectropolarimeter at 25°C.

2.2. Small-angle neutron scattering (SANS)

SANS experiments were carried out using the Bio-SANS instrument at the High Flux Isotope Reactor of Oak Ridge National Laboratory. Scattering data were recorded for scattering vectors ($q$) 0.008 < $q$ < 0.27 Å$^{-1}$ ($q = (4\pi/\lambda) \sin(\theta/2)$, $\lambda$ is the neutron wavelength and $\theta$ is the scattering angle) using 6 Å neutrons with a wavelength spread ($\Delta\lambda/\lambda$) of 0.15. The measurements were performed at room temperature in 1.0 mm path quartz cuvettes. The raw data were corrected for detector sensitivity, solvent background and transmission. The radius of gyration ($R_g$) was calculated by using the Guinier approximation. The theoretical scattering profile of the crystal structure of LHC II was calculated using the program ORNL_SAS. The quality of the fit to the data was evaluated with the reduced $\chi^2$ parameter.

3. Results

The properties of purified LHC II-TX100 were investigated using SANS. Figures 1A and B show the scattering profiles of LHC II-TX100 and free detergent in 75% and 25% D$_2$O, respectively. The free micelle and LHC II-TX100 curves overlay well in the high q region but deviate at low q. The absence of a Guinier region in the LHC II-TX100 curves in both 25% and 75% D$_2$O suggests that larger order aggregates are present in the sample. However, in the case of the free micelles, $R_g$ was determined to be 23.7±0.2 Å (inset of Figure 1A). A theoretical scattering curve, calculated from the crystal structure of plant LHC II using ORNL_SAS, was compared to the theoretical LHC II scattering curve. The counting statistics in the experimental data at high $q$ values were poor due to the low contrast between LHC II and the solvent and also the relatively low concentration of LHC II. In contrast to the fit with the LHC II-TX100 data, the simulated LHC II
crystal structure scattering curve (shown as a solid red line) overlaid well with the profile of LHC II-BOG curve ($\chi^2 = 0.38$, Figure 2B) suggesting that the protein is in its native folded state. Guinier fitting gives an $R_g$ of 28.3 ± 1.6 Å. A Guinier region is also clearly visible in the LHC II-BOG data in 100% D$_2$O, demonstrating that there are no larger order aggregates present.

![Figure 1](image1.png)

**Figure 1.** SANS data of LHC II-TX100 (open circles) and TX100 micelles (open triangles) in (A) 75% D$_2$O and (B) 25% D$_2$O. Theoretical scattering profile of LHC II crystal structure is presented as the solid red line. Panel A (inset): Guinier plot of TX100 in 75% D$_2$O.

The circular dichroism (CD) spectrum of LHC II in the visible region results from the excitonic coupling of adjacent Chls and is very sensitive to changes in the conformational state of the trimeric complex. The CD spectrum of LHC-BOG is shown in Figure 2B and is similar to previously reported spectra of the protein complex. The shoulders at 648 nm and 478 nm and a broad positive signal at 412 nm indicate that LHC II is in its native trimeric state. In comparison, no CD signal was observed.

![Figure 2](image2.png)

**Figure 2.** (A) SANS data of BOG in 100% D$_2$O (open circles) and LHC II dissolved in 1.8% (w/v) BOG in 100% D$_2$O (open triangles) and 15.5% D$_2$O (opens squares). Theoretical scattering profile of LHC II crystal structure is presented as the solid red line. (B) Circular dichroism spectra of LHC II dissolved in 1.8% (w/v) BOG (solid green line) and in 1.7% (w/v) Triton X-100 (dashed blue line) at a detergent to Chl ratio of 40:1 after 48h incubation.
for LHC II dissolved in TX100 (dashed blue line) after 48 h. This indicates that the excitonic couplings between the Chls are lost, as would occur if the Chl dissociated from the protein as result of unfolding.

Conclusions
The molecular components of the photosynthetic apparatus such as the photosystems and their associated light harvesting antennae have properties that to date have not been emulated by synthetic approaches. In order to take advantage of their exquisite properties for the development of biohybrid photoconversion devices it is necessary to develop approaches for extraction of these transmembrane protein complexes in their functional state. This study investigated the effect of TX100 and BOG on the stabilization of LHC II. Our SANS analysis shows that the LHC II-TX100 preparation is in a non-native conformation that is inconsistent with an intact trimeric complex, but instead supports the presence of larger order aggregates in the preparation. The existence of a non-native conformation of LHC II is supported by circular dichrosim spectroscopy, which demonstrated that LHC II is inactive in TX100 detergent after 48h incubation. In contrast, LHC II is monodisperse in BOG detergent and maintains its native trimeric conformation under the conditions examined in this study. This investigation provides insight into the effects of detergents on the solution structure of LHC II.

Acknowledgements
M.B.C thanks Capes-Brazil for the support. This work was supported by the ORNL Laboratory Director's Research Development Program. Research at Oak Ridge National Laboratory's Center for Structural Molecular Biology (CSMB) is supported by the Office of Biological and Environmental Research, using facilities supported by the U. S. Department of Energy, managed by UT-Battelle, LLC under contract No.DE-AC05-00OR22725.

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