

Abstract No. DiMa0146

Surfactant Behavior of Light-Harvesting Chlorophyll Protein Complex LHCII and Associated Lipids

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Beamline(s): X22B

Introduction: LHCII, the light-harvesting chlorophyll *a/b* protein complex associated with photosystem II, is noteworthy among membrane proteins for forming ordered complexes in a number of environments. LHCII forms a trimer in detergent solutions and in two dimensional crystals, and there is good reason to believe that the trimer represents the native, functional form of the complex [1]. Structural changes evidently accompany the protein's function, but this has been discerned primarily through spectroscopic methods and ex-situ assessments of optical activity. In-situ structural measurements would be extremely valuable, possibly answering such questions as whether a monomer-to-trimer transition occurs as a response to varying levels of light [2]. To study the structure of the protein in a controlled environment with a similar chemical environment to that which the protein might experience in the membrane, we investigated monolayers of the LHCII complex and of associated lipids, assembled on water surfaces.

Methods and Materials: LHCII and lipids (DGDG, PG, SQDG, and MGDG) were extracted from pea leaves (a PG sample obtained from cyanobacteria was also investigated). LHCII monolayers were spread onto a buffered subphase from a 25%v/v isopropanol solution, prepared immediately before deposition. Lipid films were spread from 1 mg/ml chloroform solutions. Surface pressures were controlled and monitored in a Langmuir trough near room temperature, and x-ray reflectivity measurements were performed under helium atmosphere using the Harvard/BNL liquid surface spectrometer at beamline X22B.

Results: The LHCII behaved as a slightly unstable surfactant. Pressure-area isotherms consistently exhibited an inflection point at surface pressures of about 25 mN/m, but with variable low-pressure slopes and small extrapolated molecular areas indicating that significant amounts of material were lost from the surface. Reflectivities measured at various pressures, Figure 1(a), could be fit by surface-normal profiles in which a dense region is evident about 10 Å from the vapor interface. Thus, most of the protein is not visible against the water background, but we can observe the associated lipid headgroups which evidently help give the complex its surfactant behavior. At higher surface pressures, this part of the protein near the interface becomes more extended. Lipids which were extracted separately were also examined at the water interface, Figure 1(b). All had uninflected pressure-area isotherms, and all could be stabilized at maximum pressures of about 50 mN/m. At the highest pressures, the model profiles clearly show the headgroup and extended hydrocarbon tails of length 20 Å, which are somewhat less extended at lower surface pressures.

Conclusions: Surfactant behavior of the LHCII complex and associated lipids has been demonstrated. However, it is not yet clear whether the ability of LHCII to undergo light-induced reversible changes is preserved in this environment. Also, in-plane ordering of both LHCII and lipids is expected, but was not observed in our experiments. We will pursue the question of in-plane ordering in future diffraction studies, as it bears directly upon the protein complex's function.

Acknowledgments: The National Synchrotron Light Source is supported under USDOE Contract DE-AC02-98CH10886.

References:

1. W. Kühlbrandt, *Current Opinion in Structural Biology* 4 (1994) 519.
2. G. Garab et al., *Biochemistry* (in press, 2002)

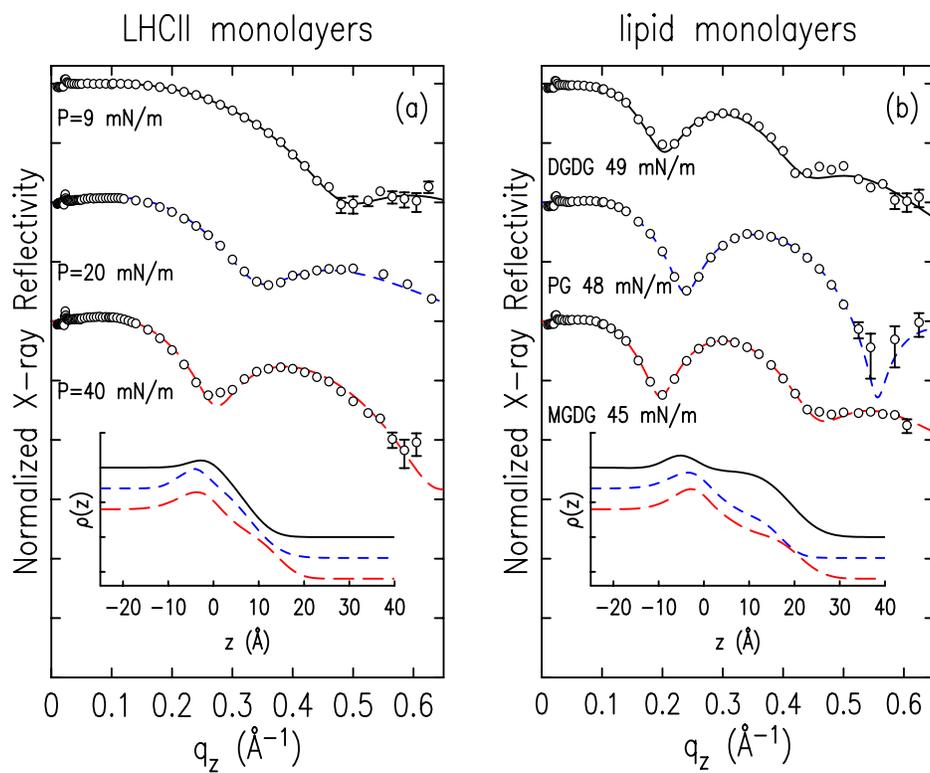


Figure 1. (a) Fresnel-normalized x-ray reflectivity measurements (shifted along log scale, circles) with fit curves (lines) for LHCII monolayers assembled on a tris buffer subphase. The corresponding real-space models (inset) show how a dense region, probably of lipid headgroups, holds the protein close to the air interface. (b) Pure lipid monolayer films: digalactosyl diacylglycerol (DGDG, from pea), phosphatidylglycerol (PG, from cyanobacteria), and monogalactosyl diacylglycerol (MGDG, from pea). At high surface pressures, these molecules assemble at the water surface with the hydrocarbon tails more or less erect, exhibiting a longer length scale than the reflectivity from the LHCII complex shows.