ESRF	**Experiment title:** Three dimensional crystals of the light-harvesting chlorophyll a/b protein complex from pea chloroplasts	**Experiment number:** LS-1465
Beamline: ID-14 4	**Date of experiment:** from:8-Nov-99 8:00 to:9-Nov-99 8:00	**Date of report:** 25-9-99
Shifts:3	**Local contact(s): Dr. Raimond RAVELLI**	*Received at ESRF:* 1- MAR 2000

Names and affiliation of applicants

Matteo Lamborghini, Werner Kühlbrandt

Max-Plank Institute of Biophysics

Heinrich-Hoffmannstr.7

D-60528 Frankfurt am Main

Germany

Report:

Light-harvesting chlorophyll a/b protein complex (LHC-II) is the major collector of solar energy in all plants. It binds about half of the chlorophyll in green plants and it is probably the most abundant membrane protein on earth. LHC-II is a trimer in the photosynthetic membrane of nearly identical monomers, each consisting of 232 amino acids. Each monomer binds and orientates a minimum of 12 chlorophyll molecules, two luteins and a neoxanthin for light harvesting and energy transfer.

Although the structure of LHC-II has been determined at 3.4 Å resolution by electron microscopy of two-dimensional crystals, this is not sufficient to allow a complete understanding of the mechanism of energy transfer from LHC-II to the reaction centre, since the effective resolution in the *z* direction is 4.9 Å. In fact the chemical difference between Chl *a* and Chl *b* (a formyl group instead of the methyl group at the 7-position in the chlorin ring of the Chl *a*) is too small to be detected at this level of resolution.

In addition the orientations of the chlorophyll tetrapyrroles have not been determined unambiguously. This is important for understanding the photochemical processes of energy trapping and transmission. This project aims at solving the structure of LHC-II at high resolution so as to fully understand the mechanism of light-harvesting and energy transfer to the reaction centre in chloroplast membranes. LHC-II was purified from pea leaves by a standard procedure. Crystals grow by vapour diffusion in hanging drops. Hexagonal plates appear in a few days at 20 °C, measuring 0.2x0.2x0.01 mm. Crystals were frozen in liquid nitrogen after harvesting with a loop from the drops. During the last experiment performed at the ID 14 (endstation EH4, ESRF, France), it is not been possible to collect a complete data set. The crystals tested showed a too high degree of mosaicity to be useful for data analysis. Moreover it has not been possible to utilise the whole time allocated to this project. At the beginning of the third shift the beam line has been shut down for a technical maintenance of the line.

More recently LHC-II has been purified both from spinach and pea leaves using a new purification protocol in order to improve the sample homogeneity.

A different strategy to obtain sufficient amount of highly homogeneous protein is the expression the LHC-II 25 KDa apoprotein in *E. coli*. After solubilisation of the inclusion body the apoprotein is bound to a Ni-column via a His-tag. Refolding of the protein, pigments binding and trimerisation occur during column elution with mixed lipid-detergent micelles (H. Rogl, K. Kosemund, W. Kühlbrandt, I. Collinson: *FEBS Letters* 432 (1998) 21-26). Crystallisation of recombinant of monomer LHC-II is possible only in presence of specific lipids composition (S. Nussberger, K. Doerr, D. Wang, W. Kühlbrandt.(1993) *J. Mol. Biol.* 234, 374-356).

On the basis of this work the energy transfer pathway from light-harvesting protein complex to the reaction centre can be solved and so make clear one of the most fascinating aspect which supports all life on earth.