EUROPEAN SYNCHROTRON RADIATION FACILITY

ESRF User Office
BP 220, F-38043 GRENOBLE CEDEX, France
Delivery address: 6 rue Jules Horowitz, 38043 GRENOBLE, France
Tel: +33 (0)4 7688 2552; fax: +33 (0)4 7688 2020; email: useroff@esrf.fr; web: http://www.esrf.fr

BAG Beam time Progress Report LS 1513

This represents a summary of the BAG progress in the reporting period, and is **in addition to** the standard ESRF report sheet for each project which will be used for the Review of the BAG.

BAG Title	Structural studies on proteins involved in energy and signal transduction
Allocation Period	**3./4. Quarter 1999**

List of publications resulting from ESRF beam time

1. Kolbe, M., Besir, H., Essen, L.-O., & Oesterhelt, D. (submitted). Structural basis for light-driven chloride transport in halorhodopsin. Science.
2. Essen, L.-O., Siegert, R., Lehmann, W. D., & Oesterhelt, D. (1998). Lipid patches in membrane protein oligomers: Crystal structure of the bacteriorhodopsin-lipid complex. Proc. Natl. Acad. Sci. USA 95, 11673-11678.
3. Essen, L.-O., Perisic, O., Lynch, D., Katan, M., & Williams, R. L. (1997). A ternary metal binding site in the C2 domain of phosphoinositide-specific phospholipase C-δ1. Biochemistry, 36, 2753-2762.
4. Essen, L.-O., Perisic, O., Roberts, M., Katan, M., & Williams, R. L. (1997). Structural mapping of the catalytic mechanism of a mammalian phosphoinositide-specific phospholipase Cδ. Biochemistry, 36, 1704-1718.
5. Essen, L.-O., Perisic, O., Cheung, R., Katan, M., & Williams, R. L. (1996). Crystal structure of a mammalian phosphoinositide-specific phospholipase Cδ. Nature, 380, 595-602.

Global Summary

Very recently, we were capable to generate a crystal form of the integral membrane protein halorhodopsin (HR). This protein is responsible for the light-driven translocation of chloride anions through the plasma membrane of *Halobacterium salinarium*. In contrast to its cousin bacteriorhodopsin with which it shares a sequence identity of 36 %, this protein promises not only to reveal conformational changes during the photocycle but also to demonstrate ion conductance using cryotrapped photointermediates of HR crystals. During the last BAG period, we collected 15 datasets on this protein at ID14-3 which culminated in the first 1.8 Å X-ray structure of HR. Although following the route of bacteriorhodopsin for obtaining structural information on early intermediates, i. e. laser excitation of HR crystals at cryogenic temperatures, we were only partially able to resolve structural changes in the vicinity of the Schiff base. On-going work will concentrate on HR mutants with altered kinetics and an improvement of the cryo-trapping conditions.

Visits made to the ESRF

Date(s) of visits	Beamline	No. of Shifts	Short Summary of each Visit
1. 2.9.-4.9.99	ID14-3	6	25 crystals of halorhodopsin which were grown in cubic lipidic phases were tested for conditions that corresponded to well diffracting crystals. 10 datasets with resolution limits between 1.8 Å and 2.5 Å were recorded under dark and light-exposed conditions (λ=532 nm). We refined the HR structure at 1.8 Å with these data. Low-temperature K-like intermediates showed that under photoequilibrium conditions the chloride in the transport site becomes apparently lost. Crystals of another archaeal rhodopsin, sensory rhodopsin II, were tested for diffraction, but proved to be useless at this stage.
2. 2.12.-4.12.99	ID14-3	6	At this period, 25 HR crystals were tested for diffraction, 5 datasets were recorded. Illumination was now done at a longer wavelength (λ=633) under different temperature, light-exposure regimes. Although focussed on obtaining the HR520 or an HR410 intermediate, the conversion ratios appear currently to be too low (<20 %) with the wild type protein. We collected a 2.3 Å dataset from catalase-peroxidase from Halobacterium salinarum in our just started efforts to build up a structural reportoire for halophilic proteins. Structure solution by molecular replacement is on the way.