ESRF

Experiment title: HIGH RESOLUTION STRUCTURES OF MEMBRANE PROTEIN CRYSTALS GROWN IN LIPIDIC CUBIC PHASES	**Experiment number:** LS 1138

Beamline: ID13	**Date of experiment:** from: 11 Nov. to 12 Nov. and 23 Nov. to: 25 Nov.	**Date of report:** 28 Feb.99
Shifts: 9	**Local contact(s):** Manfred Burghammer and Hassan Belrhali	*Received at ESRF:* 01 MAR. 1999

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Report:

The experiment was focused on one of the transition states, the M-state, of bacteriorhodopsin (bR). The determination of structural modifications between the ground state and the M-state would give us insights on the proton translocation mechanism.

Experimental conditions: 9 shifts were allocated to the bR project on the ESRF micro-focus beamline ID13. The experiments have been carried out in 2 runs: 3 and 6 shifts. The synchrotron was operating in the a 16-bunch mode for the first run and 1/3 filling mode for the second run. The electron beam value was respectively 70 mA and 120 to 160 mA. The experimental set-up allowed diffraction patterns to be recorded up to a 2.1 Å resolution limit. During the entire allocated beamtime, about 30 micro-crystals were diffracted (crystal sizes ranging from 20 to 50 microns in the larger dimension and 5 to 10 microns in the thickness).

Experimental method: During this experiment we focused on a bR mutant, D96N, known to slow down the photocycle by a factor of thousand. All crystals were grown in a lipidic cubic phase (Landau et al., 1996), mutant crystals grow systematically smaller than wild type crystals. Crystals extracted from the cubic phase are fished in a loop, illuminated under yellow light in order to excite the proteins in the M-state and flash-frozen in a 100 K N_2 gas stream, preventing the relaxation toward the ground state. The micro-crystals are then centered in the beam with the help of two optical devices mounted 90 degrees apart from one another. As the microcrystals are embedded in the lipid matrix, it is difficult to observe and align them, this is particularly the case with the yellow color of the crystals obtained in the M-state. The mounting and alignment procedures were improved by dissolving the cubic phase prior to the experiment adding a lipase. At the time of this experiment, we were not sure that the lipase treatment was harmless to the crystals, therefore both procedures were followed in parallel.

Remark:

The ground state structure of BR first determined to 2.4 Å resolution (coordinates deposited in the PDB) is now refined to 2.2 Å resolution from data collected on ID14-EH3. Data were corrected for a 34% twinning and refined using the CNS program. At the present stage the crystallisation conditions controlling the twinning are still under investigation.

Results: Some of the crystals showed a yellow color indicating a large population of proteins in the M-state, others were partly purple indicating a possible relaxation toward the ground state even at 100K (these crystals were prepared in advance). Most of them did not diffract to high resolution and we were not able to collect any useful data. In conclusion, the preparation of the M-sate from the D96N mutant crystals has to be modified, in particular in order to avoid the formation of salt microcrystals which appear during the illumination procedure. These experiments, although we were not able to collect a data set of bR in the M-state, provided new ideas on the experimental improvements which are necessary. These improvements are impossible to test on a standart diffractometer because of the very small size of the bR crystals, especially for the mutant crystals.

References:

E. Pebay-Peyroula, G. Rummel, J.P. Rosenbusch and E. MM. Landau, Science , 277, 1676 (1997).

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