Experiment title: BAG-Frankfurt, Sodium-Proton-Antiporter NhaA from *E.coli* , Methylene-tetrahydromethanopterin reductase	Experiment number: MX-135

Beamline: ID29	Date of experiment: from: 9.2.04 to: 10.2.04	Date of report: 1.2.05
Shifts: 2	Local contact(s): **Didier Nurizzo**	*Received at ESRF:*

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Report:

NhaA is the main Na^+/H^+ antiporter in *Escherichia coli*. The protein plays an important role in adaptation of the cells at high sodium concentrations and pH homeostasis. Its activity has a pronounced pH dependence. High resolution structural information for NhaA is not available. The crystals are generally smaller than 0.2 x 0.1 x 0.1 mm^3 and diffraction quality cannot be tested at home radiation sources. Crystal quality has been continuously improved to a diffraction limit of ~3.8 Å resolution. To obtain phase information selenomethionine containing NhaA was produced and crystallized. These crystals show the same properties as those from the wild-type protein. They belong to the space group P212121 with unit cell constants of a=109 Å, b=122 Å, and c=124 Å. Best diffraction was noted up to 3.7 Å, but resolution was compromised by radiation damage during data collection. Furthermore, the crystals are not isotropic. Especially the cell constant c varies by up to 8 Å. Fluorescence scans were performed and data collection for either SAD or MAD strategy was carried out with single crystals. Several crystals have been tested and attenuation level has been optimized for data collection. Following data sets have been obtained: crystal 1, peak , 4.2 Å

resolution, R_{sym} 8 %, 99 % complete; 2. crystal 2, peak, 4.4 Å, R_{sym} 10 %, 99 % complete; 3. crystal 3, inflection, 4.5 Å, R_{sym} 8 %, 97 % complete; crystal lost diffraction while measuring remote wavelength; 5. crystal 4, remote, 4.7 Å, R_{sym} 9 %, 99 % complete; inflection, 4.7 Å, R_{sym} 8 %, 97 % complete; 6. crystal 6, peak, 4.3 Å, R_{sym} 8 %, 99 % complete; inflection, 4.3 Å, R_{sym} 10 %, 100 % complete; remote, 4.3 Å, R_{sym} 13 %, 100 % complete. Data are used in combination with previously obtained data sets of native crystals for phase determination.

Methylene-tetrahydromethanopterin (H_4MPT) reductase (Mer)

Mer is an essential enzyme of the methanogenic pathway and catalyses the reduction from methylene-H_4MPT to methyl-H_4MPT by oxidizing coenzyme F_{420}. The structure of the enzyme from *Methanopyrus kandleri* was solved but substrate binding was, so far, unsuccessful. Using Mer from *Methanosarcina barkeri* cocrystallisation experiments in the presence of F_{420} and methylene-H_4MPT leaded to crystals.

Data were collected at 2.6 Å resolution. The R_{sym} of the data was 10.3 % and the completeness 97.1 %. The structure could be solved by molecular replacement and the coenzyme F_{420} was detected in the electron density. The structure was subsequently refined to an R and R_{free} factor of 18.5 % and 22.1 %, respectively. A manuscript describing the results was sent to Protein Science.

Start-up of the beamtime was delayed by 3 hours due to software problems, which were solved by ESRF staff. Software errors blocked data collection for an additional hour during the beamtime.