	Experiment title: Stockholm BAG	**Experiment number**: LS-1955
Beamline: 14:1	**Date of experiment**: from: 5/5/01 to: 7/5/01	**Date of report**: 23/8/01
Shifts: 6	**Local contact(s)**: Dr Steffi Arzt	*Received at ESRF:*

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Report:

1. NrdH

Thioredoxin, glutaredoxin and thioredoxin reductase are part of the system maintaining and regulating the proper redox state in the cell. Deficiencies in this system often are associated with human disease. In this project, we wished to determine the structure of a novel member of the glutaredoxin family, NrdH from E. coli. This protein belongs, based on sequence, to the glutaredoxin family, however has a thioredoxin-like activity profile. Attempt to solve the structure of this protein by MR have failed and we therefore performed a MAD experiment using selenomethionine substituted protein.

The crystal structure of recombinant *E. coli* NrdH-redoxin in the oxidized state has been determined at 1.7 Å resolution by multi-wavelength anomalous diffraction. Data were collected at beamline BM14, ESRF. NrdH-redoxin belongs to the thioredoxin superfamily and is structurally most similar to *E. coli* glutaredoxin 3 and phage T4 glutaredoxin. The angle between the C-terminal helix α3 and strand β4, which differs between thioredoxin and glutaredoxin, has an intermediate value in NrdH-redoxin. The orientation of this helix is to a large extent determined by an extended hydrogen-bond network involving the highly conserved sequence motif W^{61}-S-G-F-R-P-D/E^{67} which is unique to this subclass of the thioredoxin-superfamily. Residues that bind glutathione in glutaredoxins are in general not conserved in NrdH-redoxin, and no glutathione-binding cleft is present. Instead, NrdH-redoxin contains a wide hydrophobic pocket at the surface similar to

thioredoxin. Modelling studies suggest that NrdH-redoxin can interact with *E. coli* thioredoxin reductase at this pocket, and via a loop that is complementary to a crevice in the reductase in a similar manner as observed in the thioredoxin-thioredoxin reductase complex.

Publication: Stehr, M., Schneider, G., Åslund, F., Holmgren, A. and Lindqvist, Y. (2001) Structural basis for the thioredoxin-like activity profile of the glutaredoxin-like NrdH-redoxin from *Escherichia coli.* J. Biol. Chem., in press

2. Complex of class I MHC with an activating Nk cell receptor

This project aims at the structural characterization of a complex of the class I MHC molecule H-2D^d with an activating NK cell receptor, using crystallography. Crystals of the complex diffract so far to medium resolution and a complete data set was collected at beam line 14:1. The data set (30-3.35 Å resolution, completeness 98.7, R-sym 5.9) is presently being used to solve the structure of the complex with the crystal structure of H-2D^d as search model. The latter has been solved previously in the laboratory using data collected at the ESRF.

3. Pyrimidine catabolic pathway

5-fluorouracil is one of the five most-used drugs in the chemical combat against cancer, in spite of its severe side-effects. These side effects are due to the rapid breakdown of the drug in the pyrimidine catabolic pathway. The rate limiting enzyme in this pathway is dihydropyrimine dehydrogenase (DPD) and its structure of this very large enzyme (more than 1000 amino acids per subunit) has been determined previously using data from the ESRF. The studies had now been extended towards another enzyme in this pathway, β-alanine synthase. A native data set has been collected to 3.25 Å resolution. The project will require more beam time for a MAD experiment, in order to solve the structure.

Statistics for data set:
nr. of reflections (total): 219948
nr. of reflections (unique): 44521
resolution: 3.25 Å (3.43-3.25 Å)
completeness: 67.2 % (68.2%) (incomplete because of limited time)
R_{merge}: 13.4 % (33.7 %)
mosaicity: 0.56
I/σ: 4.4 (2.0)
space group: $P2_1$
unit cell: a = 120.28 Å b = 77.50 Å c = 223.95 Å β = 95.59

4. Aklavinone hydroxylase

Anthracyclines are important chemotherapeutic agents of the polyketide class of antibiotics produced by actinomycetes. In Streptomyces species, these antibiotics are synthesized by a number of enzymes, encoded by open reading frames denoted *rdmA* to *rdmF*. *RdmE* encodes a FAD-dependent monooxygenase which functions as an aromatic polyketide hydroxylase. As one step towards the elucidation of the pathway for the synthesis of this class of antibiotics, we have crystallised Rdme with bound substrate and intend to determine the structure of this hydroxylase. Due to lack of sequence homology to other hydroxylases of known structure, we have to resort to MAD/MIR techniques for phase determination. Production of Se-methionine substituted protein in Streptomyces has not yet been successful and we have to screen for heavy metal derivatives. While the crystals diffract to better than 2.5 Å resolution at synchrotrons, no useful data can be obtained using home sources. Therefore, screening for derivatives has to be done at the synchrotron.

During this visit five data sets of putative derivatives were collected.

Compound	resolution	R-sym (%)	completeness (%)	redundancy
Derivative 1	3.2	8.5	97.5	4.4
Derivative 2	3.0	9.7	96.7	5.6
Derivative 3	3.0	6.5	88.5	3.5

Derivative 4	3.0	8.4	84.0	2.3
Derivative 5	3.5	12.4	89.9	3.0

6. Enzymes of the DOXP pathway

A novel pathway for the synthesis of isopentenyl diphosphate, a fundamental building block of isoprenoids, has been discovered rather recently. Enzymes of this pathway are very promising targets for the development of new antibiotics. Native data on crystals of one of these enzymes, 1-deoxy-xylulose-5-P reductoisomerase, have been collected. Statistics of the data set:

Spacegroup P1, Unit cell: 65.89 70.46 98.75 102.57 108.94 99.55

Resolution: 2.23Å, completeness: 94.2, Rsym: 0.071