| ESRF | Experiment title: Frankfurt BAG | Experiment number: LS-1661 |
|---|---|---|
| Beamline: BM14 | Date of experiment: from: 30-Mar-00 8:00 to: 1-Apr-00 7:00 | Date of report: 24-Aug-2000 |
| Shifts: 6 | Local contact(s): Gordon Leonard | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

Björn Mamat* and Ulrich Ermler (subproject metal-free hydrognease)
Annette Roth* and C. Roy D. Lancaster* (subproject fumarate reductase)
Max-Planck-Institute of Biophysics,
Department of Molecular Membrane Biology,
Heinrich-Hoffmann-Str. 7,
D-60528 Frankfurt am Main

## 1. Towards the crystal structure of the metal-free hydrogenase from methanogens

Björn Mamat, Seigo Shima, Ulrich Ermler, and Rudolf K. Thauer

*MPI of Biophysics, Frankfurt/Germany, and MPI for terrestrial Microbiology, Marburg/Germany*

In several methanogenic archaea a novel type of hydrogenase without redox-active transition metals has been identified [1, 2]. The oxygen-sensitive, homotrimeric enzyme catalyzes the reversible, direct hydride transfer from $H_2$ to methenyl-$H_4$MPT (one of methanogens' C1 carriers). Until now, the structural base of its unique mechanism of hydrogen splitting is completely unknown.
Crystals from the purified enzyme from *Methanothermobacter marburgensis* were grown using ammonium sulfate as precipitant. They belong to spacegroup $P6_x22$ with unit cell dimensions of 138.4, 138.4, and 98.1 Å. A native data set to 2.8 Å resolution was collected at the BM14 beamline. Reflection intensities were calculated using DENZO. The processed data from 83509 reflections measured contain 13690 unique reflections and were 96.4% complete in the resolution range between 30 and 2.8 Å. The $R_{merge}$ was calculated to be 7.9% in this range. Preparation of heavy-atom derivatives for phase determination using MIR and/or MAD is in progress.

[1]FEBS Lett. 261, 112-116, 1991.
[2] Angew. Chem. Int. Ed. 37, 3300-3303, 1998.

## 2. Fumarate reductase, a membrane protein complex from *Wolinella succinogenes*

Quinol:fumarate reductase (QFR) couples the reduction of fumarate to succinate to the oxidation of quinol to quinone, in a reaction opposite to that catalysed by mitochondrial complex II (succinate dehydrogenase). QFR from the anaerobic bacterium *Wolinella succinogenes* consists of three protein subunits, FrdA, FrdB, and FrdC, with a total molecular weight of 130 kDa. FrdC (30 kDa) is a dihaem cytochrome *b*, which anchors the enzyme in the membrane and contains the site for menaquinol oxidation. FrdA (73kDa) contains covalently bound FAD and carries the site of fumarate reduction. FrdB (27 kDa) contains a binuclear [2Fe-2S], a trinuclear [3Fe-4S], and a tetranuclear [4Fe-4S] iron-sulphur centre. This enzyme is currently the best investigated system involved in anaerobic respiration.

Crystals of this bioenergetically important 130 kDa membrane protein complex diffract up to at least 2.1 Å and have two different unit cells, both of the monoclinic space group $P2_1$. The unit cell of crystal form "A" is a = 85.2 Å, b = 189.0 Å, c = 117.9 Å, and β = 104.5°. Crystal form "B" has the unit cell dimensions a = 118.4 Å, b = 85.1 Å, c = 188.9 Å, β =96.5°. There are four complexes per unit cell and thus two complexes in the asymmetric units of both unit cells. However, many crystals contain both unit cells, and single crystals of both unit cells are not distinguishable morphologically. Intensive screening of preselected crystals at the beam line is required prior to data collection. This makes data collection (beam)-time consuming, but still feasible. Using data collected earlier at ESRF BM14 (cf. experimental reports for LS-1369), the structure of crystal form A has been solved by multiple isomorphous replacement and anomalous scattering (MIRAS) and refined to 2.2 Å resolution, and that of crystal form B has been solved by molecular replacement (MR) and refined to 2.33 Å resolution.

During the beam time allocated to this subproject, two higher quality data sets (all for crystal form A) and a partial data set for a mutant QFR could be collected. Due to the optimum conditions for data collection at BM14 first established in November 1998 (cf. our previous reports for LS-1137 and LS-1369), only two crystals were required for the first data set and only one crystal each was required for the two other datasets listed below. Structures for the first two data sets are currently being refined.

**Table: *W. succinogenes* QFR diffraction data collected at ESRF BM14 (31 March-1 April 2000)**

| | resol. range [Å] | measured reflections | unique reflections | complete [%] | I/σ(I) | >2σ [%] | $R_{sym}$* [%] |
|---|---|---|---|---|---|---|---|
| *inhibitor complexes* | | | | | | | |
| proj26/350c604_1 | 40.0-1.99 | 792,544 | 229,426 | 93.1 | 8.1 | 63.4 | 9.4 |
| /348c606_1 | *2.04-1.99* | *33,870* | *14,638* | *89.2* | *1.5* | *27.6* | *45.9* |
| proj33/404x02 | 40.0-2.80 | 279,256 | 87,157 | 98.1 | 8.1 | 80.0 | 9.7 |
| | *2.87-2.80* | *18,600* | *5852* | *99.2* | *3.0* | *54.3* | *33.2* |
| *mutant (partial data set)* | | | | | | | |
| mut1/377c603_1 | 30.0-2.90 | 65,960 | 42,480 | 53.4 | 7.8 | 34.8 | 9.4 |
| | *2.97-2.90* | *2,897* | *2407* | *45.5* | *2.4* | *23.4* | *30.1* |