ESRF	**Experiment title**: Low-spin sulfite reductase from *Desulfovibrio vulgaris* Hildenborough - Determination of the three-dimensional structure using the MAD method near the Fe K absorption edge	**Experiment number**: LS-1152
Beamline: BM-14	**Date of experiment**: from: 14-12-1998 07h00 to: 17-12-1998 07h00	**Date of report**: 24-2-1999
Shifts: 9	**Local contact(s)**: Andrew Thompson	*Received at ESRF:*

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Report:

Previous tests had shown that the crystals of sulfite reductase, grown at 4°C, were very sensitive to temperature, therefore they were transported to the ESRF at 4°C and stored in a cold room near the beam line prior to the experiment. The crystals (with typical dimensions between 0.1 and 0.2 mm) were frozen inside the cold room using liquid nitrogen and then transported to the beam line and placed in the goniometer head using an extended arc device from Oxford Cryosystems™. An edge scan was successful in revealing the Fe K-edge from a single crystal. However, careful analysis of the first diffraction images obtained on a MAR 345 imaging plate system revealed the crystals to be twinned, which prevented us from carrying out the full MAD experiment. Nevertheless, a set of images corresponding to a 180° ϕ-range was recorded at the peak wavelength. Work in progress is aimed at processing these images and separate the twin components. Also, a search for other crystal forms will be carried out. This task is made difficult by the fact that the protein is purified in very small amounts from the cells of *Desulfovibrio vulgaris* Hildenborough.

The residual time from this experiment was then used for several single-wavelength experiments:

1. Crystals of a presumed complex between [NiFe]-hydrogenase and the nine haem cytochrome *c* from *Desulfovibrio desulfuricans* ATCC 27774 were obtained, under anaerobic conditions, using the vapor diffusion method. They appeared, as hexagonal bipyramids, after 15-25 days. One crystal (approximately 0.2x0.2x0.2 mm^3) was frozen and a data set collected at λ=0.886 Å to an effective maximum resolution of 2.3 Å (R_{merge} = 13.4%, completeness 69.5% and redundancy 7.1). The crystal belongs to hexagonal space group $P6_122$ or $P6_522$, with cell dimensions a= 159.0 Å, c=166.7 Å, γ=120.0°. The crystal used in this data collection was successfully stored in a Dewar vessel and later a second data set was collected on ID14-EH3 (see LS-1153 Experimental Report for more info).
2. Reduced crystals of nine haem cytochrome *c* from *Desulfovibrio desulfuricans* ATCC 27774 were obtained *in situ* by adding sodium dithionite to the oxidized crystals, after transferring them to a set of new crystallization drops at different pH values. Two data sets, from crystals reduced at different pH values (7.5 and 9.5) were collected at λ=0.886 Å, under cryogenic conditions. The first data set, from a crystal reduced at pH 7.5, extended to a resolution of 2.0Å (R_{merge} = 6.4%, completeness 97.6% and redundancy 2.7). The second data set, from a crystal reduced at pH 9.5, extended to a resolution of 2.2Å (R_{merge} = 8.0%, completeness 99.8% and redundancy 3.1). All the crystals belong to the same $P2_1$ monoclinic space group as the oxidised crystals, with two molecules in the asymmetric unit and very similar cell parameters. The phase problem was solved by molecular replacement, with the program AMoRe, and using as a search model the three-dimensional structure of the oxidized nine haem cytochrome *c* at pH 5.5. The solution obtained for the reduced crystal at pH 7.5 had a correlation coefficient of 70.1 and an R factor of 31.2. Refinement of the crystal structure, using program X-PLOR, is in progress. Current values of R_{free} and R are 32.2% and 29.3%, respectively. A second data set from a crystal reduced at pH 9.5 was later collected at ID14-EH3 and used to solve the structure (see LS-1153 Experimental Report for more info). These experiments aim at exploring the Redox-Bohr effect in this cytochrome by X-ray diffraction an modelling methods.