ESRF	**Experiment title**: High resolution data collection from crystals of Hydrogenase from *Desulfovibrio desulfuricans* ATCC 27774 at 100 K to improve the atomic model**.**	**Experiment number**: **LS-1153**
Beamline: ID14-EH3	**Date of experiment:** from: 16-12-1998 07h00 to: 17-12-1998 07h00	**Date of report:** 24-2-1999
Shifts: 3 (BAG)	Local contact(s)**:** Laurence Dumon	*Received at ESRF:*

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Report:

No suitable crystals of [NiFe] Hydrogenase from *Desulfovibrio desulfuricans* ATCC 27774 could be obtained that would lead to higher resolution data than what was already available (1.8 A). Therefore, since this proposal was included in a BAG with LS-1093 and there was residual time available, it was used to carry out the following 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. One crystal (approximately 0.2x0.2x0.2 mm^3) previously used to collect a 2.3 Å resolution data set on BM-14 and then stored in a Dewar vessel (see LS-1152 Experimental Report) was used to collect a data set at λ=0.935 Å to 2.0 Å resolution (R_{merge} = 7.2%, completeness 99.8% and redundancy 9.3).

Several trials were made to solve the phase problem by the molecular replacement method using as search models, the three-dimensional structures of both components of the complex ([NiFe]-hydrogenase and nine haem cytochrome *c*). These trials were unsuccessful and this is probably due to the fact that these crystals do not contain the complex, as initially thought, but rather a different protein. In fact, the crystals were obtained from a rather impure batch of protein, because further purification of this batch, under anaerobic conditions, led to complex dissociation. Work aiming at characterising this protein is in progress, but is greatly hampered by the fact that crystallization is the only means available of isolating it from the batch, and only one or two small crystals can be obtained from each drop, after 15-25 days.

2. Reduced crystals of nine haem cytochrome *c* from *Desulfovibrio desulfuricans* ATCC 27774 were obtained *in situ* at pH 9.5 by adding sodium dithionite to the oxidized crystals, after transferring them to new crystallization drops at this pH value. One data set was collected at λ=0.935 Å, under cryogenic conditions to 1.8 Å resolution (R_{merge} = 3.4%, completeness 95.1% and redundancy 3.4). This crystal belong to the same monoclinic $P2_1$ 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, using as a search model the three-dimensional structure of the oxidized nine-haem cytochrome *c* at pH 5.5. The solution obtained had a correlation coefficient of 66.6 and an R factor of 31.9. Refinement of the crystal structure, using program X-PLOR, is in progress. Current values of R_{free} and R are 33.6% and 31.1%, respectively. Another data set from a reduced crystal at pH 9.5 had been previously collected at BM-14 but was not used, since it only extended to 2.2 Å resolution (see LS-1152 Experimental Report for more info). These experiments aim at exploring the Redox-Bohr effect in this cytochrome by X-ray diffraction an modelling methods.