ESRF	**Experiment title:** MAD experiments on a new type of Fe-only hydrogenase from Desulfovibrio vulgaris	**Experiment number:** LS-899
Beamline: BM14	**Date of Experiment:** from: 23-Jan-98 to: 26-Jan-98	**Date of Report:** Aug-98
Shifts: 9	**Local contact(s) :** Gordon LEONARD	Received at ESRF: 01 SEP. 1998

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Report:

Crystals of HDH were successfully frozen to 100K, using a mixture of glycerol and maltose as cryo-protectant. A fluorescence spectrum was then measured from the crystal. This spectrum confirmed the presence of Fe ions in the crystals, and allowed the choice of wavelengths at which the MAD data were to be collected. Figure 1 shows the XANES spectrum.

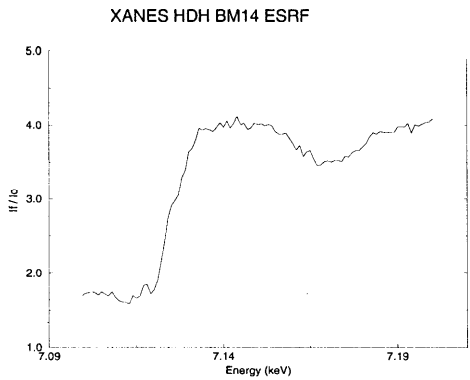

Figure 1

The space group and unit cell dimensions were confirmed from a preliminary exposure to be $P4_{1(3)}2_12$, a=b=131Å c=119Å.

From previous experiments at the home X-ray source, it was known that only few crystals of HDH diffract to medium resolution. Twenty crystals were tested during this experiment, but unfortunately none diffracted beyond 8Å.

Several crystals from which diffraction to about 6Å had been observed in the laboratory, had been shipped to the ESRF frozen in liquid nitrogen. These crystals did not diffract beyond 5Å. HDH contains iron-sulphur clusters, where the distance between the Fe ions is in the order of 3Å. Diffraction to only low resolution would not therefore provide useful information on the redox mechanism of the protein.

It had been hoped that the use of the intense X-ray beam available on BM14 would have improved the resolution limit already seen at the home source. However, this was not the case, and indicated therefore that the protein molecule is flexible leading to a disordered crystal structure.

In view of the poor results, and with no further crystals available, the experiment was abandoned.

As a backup project, crystals of Lama VH anti RR6 (40μm x 40μm x 300μm) were mounted on the beamline. These crystals were of space group $P3_221$ with cell dimensions a = b = 46.7Å, c = 121.1Å. Two data sets of 180^o were collected at room temperature with an exposure time of 30 s per degree, see table 1.

Data collection	
total number of observations	96153
number of unique reflections	13978
overall % data > 3σ (last shell)	76.5 (78.6)
overall R-merge (%) (last shell)	6.3 (9.8)
overall I/σ I(last shell)	9.3 (5.3)

Table 1

The structure of the Lama fragment was determined by molecular replacement, using a search model of camel VH anti-lysosyme. The model was then refined with XPLOR, slow cooling and energy minimisation, using all data between 10 and 2.481. The final R_{work} was 20.7% (R_{free} = 28.9Å).

A publication of this work is presently in preparation.