ESRF	**Experiment title:** Split soret - a cytochrome c from D. Desulfuricans ATCC 27774 - Determination of the three-dimensional structure using the MAD method near the Fe absorption edge	**Experiment number:** LS-397
Beamline: D14-BL19	**Date of experiment:** from: 09-FEB-96 07:OO to: 11-FEB-96 23:00	**Date of report:** 23-May-96
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Report:

Crystals of SSC were obtained in space group P21212i with cell dimensions a=98.2A b=110.6 A, c=100.5 A, $\alpha=\beta=\gamma=90°$. Diffraction data were collected using a 180 mm MAR scanner from a small (0.5x0.3x0.3 mm^3) frozen crystal of SSC at three suitable wavelengths near the Fe absorption edge. A fourth data set was measured at λ near 1 A. The diffraction images were processed with DENZO and the resulting intensities scaled with SCALEPACK in such a way as to preserve the multiple observations of all the measured Bijvoet mates. The CCP4 program suite was then used to merge the scaled data (ROTAPREP / AGROVATA / TRUNCATE) and to scale together the different wavelength data (SCALEIT).

The anomalous difference Patterson map calculated from data with maximal anomalous differences (λ_2 1.7417 A) showed clear peaks which could be interpreted using RSPS in terms of three of the expected Fe-sites.

The remainder of the Fe sites were located by means of successive anomalous Fourier syntheses using as phases the refined protein phases (MLPHARE) from the previously known and refined sites. A total of 8 Fe sites were found, consistent with four monomers in the asymmetric unit. Their positions were used to derive the non-crystallographic symmetry operations between the independent molecules (LSQKAB). Phase refinement with MLPHARE in the resolution range 10 2 d ≥ 3.0 A converged to an overall f.o.m. of 0.58.

Density modification, averaging, solvent flattening and phase extension (20 ≥ d ≥ 2.5 A) procedures (DM) produced a much improved electron density map. A polyalanine model was built into this map, corresponding to most of the polypeptide chain trace. This model was used for calculating phases which were combined (SIGMAA) with the original MAD/MLPHARE phases (10 ≥ d ≥ 3.0 A) to produce a set of phases with an overall f.o.m. of 0.80. The density modification, averaging, solvent flattening and phase extension (20 ≥ d ≥ 2.5 A) procedures (DM) were then used to further improve the electron density map. This map allowed a very nearly complete trace of the electron density and a polyalanine model containing 232 residues was built. This number of residues is reasonably consistent with the monomer molecular weight of 26.3 KDa and results in a unit cell solvent content of about 40%.

The aminoacid sequence of this protein is not yet available and therefore a more complete model building and verification has not yet been possible.