ESRF	**Experiment title:** Structure determination of a novel thioredoxin-like protein from Bradyrhizobium japonicum	**Experiment number:** LS 678
Beamline: BM02	**Date of Experiment:** from: 22 Jan. 1997 to: 23 Jan. 1997	**Date of Report:** 12 March 98
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Report:

TlpA is a membrane-anchored thioredoxin-like protein isolated from Bradyrhizobium japonicum. The molecular weight of TlpA (23,260 D) is roughly twice that of the known bacterial thioredoxins. There is a 34% identity (51% similarity) in the common regions, which are distributed over 88% of the TlpA sequence (TlpA has 3 inserts plus an N-terminal extension). A solubilized form of TlpA ("TlpAsol") lacking the 37 amino acid N-terminal membrane anchor was over-expressed in E. *coli* and crystallizes in space group P212121 with unit cell dimensions of about 52 x 77 x 85 A. The needle-like crystals (about 50 x 100 x 700 μ) can be flash-cooled in liquid nitrogen.

In the 24h period assigned for this project high (2.3 A) and low (3.2 A) resolution native data sets were taken, plus low resolution data sets of 4 potential heavy atom derivatives. The crystals had been prepared and flash cooled in advance and brought to the esrf in liquid nitrogen. Transfer to the goniometer on BM02 was done using our own equipment. The wavelength available ($\lambda = 1.069$Å) was appropriate for the measurement of the anomolous component at the L-III edge of platinum (1.0722A).

Summary of data sets taken at BM02, 22 - 23 January, 1997

Derivative	time sec	Max res. A	Rmerge (xds) %	completeness %
native 1	10	2.33	8.7	89 (98% to 2.6A)
native 2	30	2.33	10.2	89 (94% to 2.6A)
native 3	3	3.2	4.6	98
EMTS	3	3.2	3.9	98
mercury acetate	6	3.2	4.5	99.8
PtC14	8	3.2	4.4	99.2
transchloroplatinate	15	3.2	6.5	99

* EMTS=ethyl mercury thiol salicylate

Results:

The 4 heavy atom derivatives, prepared by soaking, were characterized by many weakly occupied sites, and only the transchloroplatinate (soaked for 28d in a saturated solution) ultimately proved to be useful. Further data was collected later at BM01 with other heavy metal soaks, and also a Xe derivative (prepared with the kind assistance of Y. Montet, LCCP/IBS, Grenoble). As the derivatives prepared with standard soaking techniques gave disappointing results suggestive of poor access to the interior of the crystal, TlpA derivative crystals were also prepared by co-crystallization with some of the same solutions used for the soaks. The co-crystallizations, although yielding visually good crystals with the same space group, generally had axis lengths changed by up to several percent. The other derivatives prepared by soaking were again characterized by many weakly occupied sites. Crystals soaked in 30mM trimethyl lead acetate or a 9mM mercury acetate solution were found to complement the transchloroplatinate derivative and improve the phasing, but only the transchloroplatinate derivative had a phasing power greater than 1. Combining all four derivatives, MLPHARE yielded an overall figure of merit of only 0.52 to 5Å (with a total of 38 sites, and including the anomolous contributions for the platinum and mercury derivatives). Direct viewing of the map with "O" didn't reveal any obvious structural features, nor could potential helix or beta-sheet elements be identified with "essens",

Given the relatively high sequence similarity with the known thioredoxin structure, we searched for molecular replacement solutions using both XPLOR and AMORE, but no solutions were found.

Work on a structural solution is continuing. Better labelling of the active-site cysteines may be achieved by chemical reduction before presentation of mercury compounds, although there is a danger of destruction of a structural S-S bond. An alternative path could lie in the collection of very high resolution native data. We have seen diffraction to beyond 1.4 Å with these crystals. If data could be collected to at least l.2 Å then developments in direct methods may soon allow a solution without derivative data.