ESRF	**Experiment title:** **Ongoing structure determination of Sgt1, a Skp1-interacting protein**	**Experiment number**: **LS1933, LS2183**
Beamline: ID14-2 ID14-2	**Date of experiment**: from: 25-01-02 to: 26-01-02 (LS1933) 17-07-02 to: 18-07-02	**Date of report**: 5 September 2002
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Report:

Background

The ~350 residue Sgt1 protein is conserved from yeast to man. The protein was originally identified as a 2-hybrid ligand of Skp1, a component of the SCF Ubiquitin ligase complex and of the yeast CBFIII kinetochore complex. Although these findings were supporting the view that Sgt1 is participating in the SCF, recent studies fueled the notion that this protein may be part of a chaperone complex required for the folding of SCF and other protein complexes. We generated several constructs to express recombinant versions of the Sgt1 protein. We have obtained crystals of a subdomain containing 3 tetratricopeptide (TPR) repeats, and are currently attempting to raise crystals of other portions of the protein.

Results

The TPR crystals are extremely sensitive to oxidation, and they are rather difficult to handle, often growing as rather fragile stacked plates at high concentrations of high-molecular weight PEG. Finding reliable criopreservation conditions for these crystals proved rather tricky as well. This has somewhat delayed the progress of this project. Recently, however, we were able to reduce the negative influence of these factors, and we managed to collect a very high resolution native dataset (1.4 Å). The Sgt1 segment included in the crystals contains only one methionine, which may be insufficient for Se-Met MAD/SAD phasing. Thus, we preferred to attempt a classical heavy atom phasing strategy. During our visit at the ESRF, we collected a dataset from a putative Hg derivative showing a very significant change in unit cell dimensions relative to the crystals of the native protein. We are currently trying to establish if the anomalous signal of this dataset is sufficient for SAD structure determination. If this turned out not to be the case, a full-fledged MAD phasing experiment at the Hg edge will be required.

Summary of collected data

Sgt1 native (High res)	**Sgt1 native (**low res)	**Sgt1 Hg derivative**
Space Group C2	Space Group C2	Space Group C2
Unit cell (Å) a=43.2	Unit cell (Å) a=43.2	Unit cell (Å) a=56.8
b=143.1	b=143.1	b=56.7
c=42,5	c=42,5	c=70.6
β=95.5	β=95.5	β=95.5
Resolution (Å) 40.0-1.4	Resolution (Å) 35.0-3.0	Resolution (Å) 35.0-2.3