ESRF	**Experiment title:** Protein crystallographic study of tropomyosin from lobster muscle.	**Experiment number:** LS530
Beamline: ID2(BL4)	**Date of experiment:** from: 28.JUL.96 15.00 to: 29.JUL.96 23.00	**Date of report:** 29-AUG-96
Shifts: 4 shifts	**Local contact(s):** Dr. Bjarne Rasmussen	***Received at ESRF:***

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Report: 1. Summary of our paper in press in *FEBS Letters.*

A new form of muscle tropomyosin crystal has been obtained, by employing new strategies in protein preparation and crystallization. Non-polymerizable tropomyosin was prepared by removing eleven amino acids at the C-terminus. The truncated tropomyosin was expressed in Sf9 insect cells by use of the baculovirus-based expression system, to obtain highly homogeneous protein preparations. By routinely monitoring homogeneity by mass spectrometry, we found that the homogeneity played a key role in obtaining good crystals. The crystal quality was also dependent on isoforms; the crystals raised from a slow muscle-specific isoform diffracted to a higher resolution, compared with a fast muscle-specific counterpart. For crystallization, a high concentration of organic solvent was used as the precipitant; in the presence of 35 % DMSO, tetragonal crystals were formed, which belong to space group $P4_{3(1)}2_12$ with cell constants of $a = b = 105.6$ Å, c = 506.9 Å. The crystals gave rise to reflexions the intensities of which were characteristically determined by the transform of a-helical coiled-coil. Thus in the region 10 to 5.5 Å resolution along c^*-axis, the reflexions were weak. For accurate measurements of these reflexion intensities, beam-line ID2 in ESRF Grenoble was advantageous owing to the high brilliance and a low background. There the crystals diffracted to beyond 3.0 Å along c^*-axis, whereas along the a^*-b^* -plane reflexions were limited to 6.6 Å. Data analysis is underway on a data set from a $PtCl_4$ derivative.

The X-ray data presented in this paper are those collected during the previous session at ESRF, LS424 from 20-Mar to 22-Mar-96.

2. Summary of the results obtained during this beam-time.

During this beam-time, we concentrated on measuring crystals of heavy atom derivatives.

There is difficulty specifically associated with this project. Reflexions between resolutions of 10 Å to 5.5 Å are so weak that, using a laboratory source, we have no chance to compare the intensities from a derivative and those from the native crystals. Therefore derivative searches had to be done during the beam time.

Among several derivative crystals measured, the following two crystals gave rise to the data sets which are satisfactory in terms of the isomorphism (with minimal changes of lattice parameters), the preservation of the ordered crystal lattice (with a reasonable Rmerge and a completeness) and the extent of intensity changes (with a reasonable χ^2 deviation). These analyses have been undertaken using software packages DENZO and SCALEPACK with a post-refinement of the lattice parameters and by taking anomalous differences into account.

Table Summary of selected data obtained from heavy atom derivatives

crystals (heavy atoms)	resolution	complete-ness (%)	Rmerge (%)	χ^2	χ^2 (deriv./nat.)	lattice const. (% change)
tms_pt_07	up to 6.6 Å	71.3			10.08	a=b=105.3 (-0.28%)
($PtCl_4$)	up to 3.5 Å	23.5	10.8	1.01	5.895	c=507.73 (+0.2%)
tms_hgi4_02	up to 6.6 Å	69.4			10.52	a=b=104.8 (-0.76%)
(HgI_4)	up to 3.5 Å	28.8	9.8	0.98	5.17	c=505.1 (-0.35%)

Measuring conditions: Beam size, 0.1 mm nominal; wavelength, 0.99 Å; detector, MAR30cm; crystal-detector distance, 500 mm; spindle axis, vertical to synchrotron; rotation angle, 3.0 deg; exposure time, 20 sec.

These results likely indicate that we have obtained two good heavy atom derivatives. It may be the case, as we expected, that our crystals could be easily labelled with a variety of heavy atom moieties. This could be accounted for by the extended surface of tropomyosin molecules, by the loosely packed crystals, by the presence of large side chains on the molecular surface, particularly with many negatively charged side chains, and by the presence of 35% of DMSO (a low dielectric constant) in the crystallization solution.

However, the difference Patterson maps calculated from these data and the native data have not given rise to well defined peaks of heavy atoms. At present we are investigating reasons for this and trying to improve scaling of the intensities. We would need to have better (with a higher accuracy) data sets from the native crystals and more heavy atom derivatives.