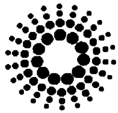

ESRF

Experiment title: Structure of orthorhombic crystals of the helical subfilaments formed by the *Ascaris* motile major sperm protein (MSP)	**Experiment number:** LS800

Beamline:	**Date of experiment:**	**Date of report:**
BM14	from: 15-DEC-97 to: 16-DEC-97	21 -AUG-98
Shifts:	**Local contact(s):**	*Received at ESRF:*
3 shifts	Dr Wim Burmeister	2 8 AOUT 1998

Names and affiliations of applicants (* indicates experimentalists):

Dr Murray Stewart
Dr Airlie McCoy (*)
Dr Anne Baker (*)
Dr David Owen (*)
Mr Richard Bayliss (*)

MRC Laboratory of Molecular Biology
Hills Rd., Cambridge CB2 2QH,
ENGLAND

Report:

Note on beamtime allocation:

We were allocated 3 shifts on BM14 for project LS800 in the initial round of beamtime allocation, and were subsequently offered 5 shifts on ID14-EH3 for project LS840, with only one week's notice, immediately prior to the time allocated for project LS800. However, we were not able to produce crystals of the macromolecular complex between Nuclear Transport Factor 2 (NTF2) and Ran at such a short notice. After consultation with Dr W. Burmeister, it was agreed that we should bring instead crystals of the dimerization domain of the *Dictyostelium* gelation factor (ABP120) and of the N-terminal domain of the Ras GTPase activating proteins and use these instead of the NTF2-Ran complex. Moreover, because we needed to collect a MAD data set for the ABP120 crystals, it was decided to use BM14 for these crystals and to instead collect the data for the motile major sperm protein (MSP) crystals on ID14-EH3.

Structure of a dimerising fragment of the rod domain of the Dictyostelium Gelation Factor (ABP 120)

The *Dictyostelium* gelation factor (ABP120) is an actin crosslinking protein that is constructed from an N-terminal globular actin-binding domain and a C-terminal rod domain constructed from 6 tandem repeats of a loo-residue motif. It is vital for its actin crosslinking function that ABP120 dimerizes and this activity depends on interaction between the rod domains. In collaboration with Drs. A. Noegel and P. Fucini (MPI, Martinsried), we obtained crystals of a bactirially-expressed construct corresponding to rod motifs 5 and 6, which retained the capacity to dimerize. these crystals had space group P212121 and unit cell dimensions a=44 Å, b=103 Å, c=124 Å and diffracted past 2 Å. However, all attempts to solve the structure by MIR had been unsuccessful because all heavy atom compounds we investigated bound to the same site in the crystals and, moreover, caused extreme non-isomorphism. The time at ESRF enabled us to perform a MAD experiment, collecting data from a mercury derivative of the crystals at three wavelengths. These data gave a traceable electron density map and the resulting structure has been refined to 2.2 Å resolution. The reason for the non-isomorphism of the derivatives is now clear: the heavy atom compounds bind to a cysteine at the hinge between two domains, and cause a rigid body shift of the domains. The structure shows that the 100-residue motifs generate an Ig fold and that dimerization in the ABP120 rod domain results primarily from an interaction between motif 6 from each chain. Motif 6 lacks one beta strand of the Ig fold and the dimer results in beta sheets from each motif joining across the interface. This structure has a considerable significance for other actin binding proteins such as filamin and ABP280 which are closely correlated with melanoma prognosis. A manuscript describing this work is in the final stages of preparation.

The work on the structure of orthorhombic crystals of Ascaris MSP is now described in the report for LS840.