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ESRF

BAG Beam time Progress Report

This represents a summary of the BAG progress in the reporting period, and is **in addition to** the standard ESRF report sheet for each project which will be used for the Review of the BAG.

BAG Title **BAG Barcelona** - Plasmid conjugative protein NΔ70 TrwB complexes

Allocation Period **Sept. 1999 – Feb. 2000**

List of publications resulting from ESRF beam time

In preparation.

Global Summary

Genetic evidence shows that TraG-like proteins, the best conserved proteins among plasmid conjugative transfer systems, are involved in the connection between relaxosome and DNA transport complex. They are hence known as "coupling proteins". The available biochemical studies are scarce, presumably because of the inherent difficulties in the purification and analysis of integral membrane proteins. TrwB is the coupling protein of conjugative plasmid R388 and its cytoplasmic domain is expected to interact with the R388 relaxosome. A plasmid was constructed that encodes the soluble domain of protein TrwB (called TrwB□N70) by deletion of the N-proximal transmembrane segments. TrwB□N70 could be overexpressed and purified as a soluble protein. The purified protein bound tightly a fluorescent ATP analogue, TNP-ATP, with a K_S = 8.7 μM, in accordance with the ATP-binding signature in its amino acid sequence, but did not show measurable ATPase or GTPase activity. A single ATP binding site was found per TrwB monomer.

We have managed to crystallize and solve the structure of TrwB□N70 in two crystal forms, monoclinic and trigonal, each 12 and 6 units, respèctively, of this 48-kDa monomer in the asymmetric unit. Native data are available for the trigonal form (to 2.4 Å resolution) and the monoclinic cell (to 2.5 Å resolution). Initial phases from a $Ta_6Br_{12}^{2+}$-derivative MAD experiment carried out at DESY for the trigonal crystals rendered some initial phases to 5-4.5 Å that finally have permitted us to localize the local symmetry operators, whose value of the rotation matrix had been established by self rotation calculations, and solve the structure applying averaging, density modification and phase extension techniques.

During the last visit to ESRF, we managed to collect data from complexes of TrwB with the ATP-analog ADP-N-P employing a trigonal (cocrystallization) and a monoclinic crystal (soaking). These data are currently being processed, although an initial omit map from the first dataset reveals the (weak) presence of the inhibitor.

We request in the enclosed application form beamtime to collect further ATP-analogs in complex with TrwB.

Visits made to the ESRF

Date(s) of visits	Beamline	No. of Shifts	Short Summary of each Visit
1. 23-24.2.2000	ID14 2	3	One complex of TrwB/ATP-analog ADP-N-P in the trigonal spacegroup and one in the monoclinic. Experiment LS-1666.