



	Experiment title: Plasmid coupling protein TrwB Δ N70.	Experiment number: LS-1666
Beamline: ID 14-4	Date of experiment: from: 9.06.2000 to: 11.06.2000	Date of report:
Shifts: 6	Local contact(s): Sean McSweeney	<i>Received at ESRF:</i>

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Report:

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 Δ N70) by deletion of the N-proximal transmembrane segments. TrwB Δ N70 could be overexpressed and purified as a soluble protein. The purified protein bound tightly a fluorescent ATP analogue, TNP-ATP, with a $K_S = 8.7 \mu\text{M}$, 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 succeeded in crystallizing and solve the structure of TrwB Δ N70 in two crystal forms, monoclinic and trigonal, each 12 and 6 units, respectively, 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 $\text{Ta}_6\text{Br}_{12}^{2+}$ -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 this visit to ESRF, we managed to collect data from complexes of TrwB with the ATP-analog ADP-N-P employing a trigonal (cococrystallization) and a monoclinic crystal (soaking). These data were absolutely unprocessable despite displaying excellent diffraction patterns. Data from crystals of similar quality collected at another synchrotron were easily processable. A problem associated with wavelength instability, problems with the detector, etc. is suspected for this beamline.