	Experiment title: Quorum-sensing transcriptional regulator TraR in complex with its negative regulator TraM and structure of TraM	**Experiment number**: MX129
Beamline: ID29 ID29	**Date of experiment**: from: 26/04/2003 to: 26/04/2003 from: 22/06/2003 to: 22/06/2003	**Date of report**: 8 August 2003
Shifts: 2 (ID29) 1 (ID29)	**Local contact(s)**: Dr. Bill Shepard	*Received at ESRF:*

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Report:

Quorum-sensing is a form of bacterial cell-cell communication, used by both Gram-positive and Gram-negative bacteria to regulate a variety of physiological functions. This mechanism is based on the production, the release and the response to small signal molecules, called autoinducers or pheromones, produced by bacteria themselves. Conjugal transfer of *Agrobacterium tumefaciens* Ti plasmids is regulated by quorum-sensing via the LuxR-type transcriptional regulator TraR and the oxooctanoyl-L-homoserine lactone, the *Agrobacterium* autoinducer. Recently, three-dimensional structure of the quorum sensor TraR bound to its autoinducer and target DNA has been solved in our lab. The structure shows that the autoinducer molecule plays a key role for the correct folding of the nascent protein instead of acting as an allosteric effector.

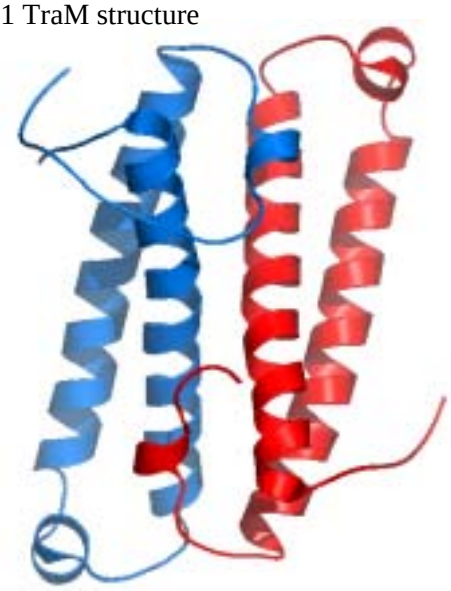
In contrast to most other LuxR-type transcriptional regulators, the activity of TraR is under the influence of the Ti plasmid encoded TraM regulator, a protein thus far only identified in *Agrobacterium tumefaciens* and other members of the family Rhizobiaceae. TraM acts as an anti-activator, forming an extremely stable inhibitory complex with the transcriptional regulator TraR, preventing the latter from recognizing its target DNA operators and, as a consequence, preventing the activation of specific gene expression. Directed mutational analysis of TraM identified a number of amino acids that play important roles in the inhibition of TraR, clustering in two region of the protein. This inhibition is absolutely required for the normal operation of the entire quorum-sensing pathway. Therefore, TraM plays a key role in determining the threshold level of the bacterial population, what is called a quorum, required for initiating the Ti plasmid conjugal transfer.

In this scenario, structural studies of TraM regulator and its complex with TraR, could greatly enhance our understanding of the molecular basis of the quorum sensing in *Agrobacterium tumefaciens*, the bacterium responsible for crown gall disease in plants.

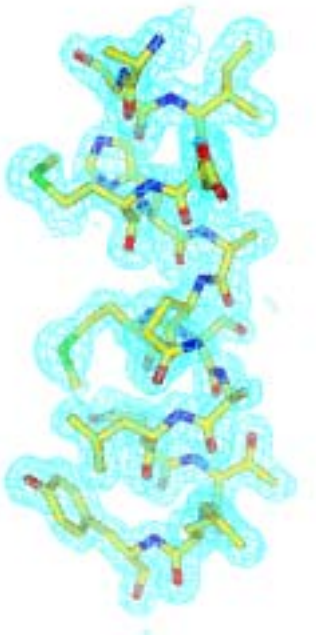
We have overexpressed, purified and characterized the recombinant regulator TraM from *Escherichia coli*. Furthermore, we have crystallized both the native and the seleno-methionine substituted protein. Because at present no relevant sequence homology has been detected with other proteins of known structure, we applied the multi-wavelength anomalous diffraction method using selenium atoms as anomalous scatterers to obtain initial phases. In particular, we have collected a complete two-wavelength MAD data set to 2.0 Å resolution at beamline ID29, that allow us to solve the structure. Furthermore, a native high resolution data set has been collected at the same beamline extending the resolution up to 1.65 Å. Refinement of the structure is ongoing. A crystallization paper has been submitted and a structural paper is in preparation.

The complex of TraR/TraM has been successfully reconstituted, purified and characterized. Crystals have been obtained but diffract poorly (7 Å). We plan to continue screening new conditions for the entire complex, as well as try to generate more compact versions of this complex by mutagenesis and/or mild trypsinization.

Fig. 1 TraM structure



Ribbon representation of TraM dimer viewed down its 2-fold axis



Electron density map at 1.8 Ang resolution (1 σ)

References:

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