Summary

The aim of this work was the determination of the three dimensional structure of the bacterial ε,ζ Toxin / Antitoxin complex (TA system) encoded by the plasmid pSM19035 of *Streptococcus pyogenes*. The described structural model is the first reported structure of a TA complex. X-ray diffraction data on crystals of a selenomethionine - derived protein (max. resolution: 3.10 Å) and the wild-type protein (max. resolution: 1.95 Å) were collected using synchrotron sources and the phase problem was solved utilising the Multiple wavelength Anomalous Diffraction (MAD) method.

The genes encoding the antitoxin ε and toxin ζ were transformed into *E. coli* and the proteins overproduced in methionine or selenomethionine containing media. A protocol for the purification of the protein complex was developed and optimised. Homogeneous protein complex ε,ζ was crystallised and X-ray diffraction data collected. Following solution of the phase problem with MAD, a structural model was built and refined. Additionally, the phase problems of two further polymorphous modifications were solved and the structures refined. Apparent conformational changes were confirmed as effects of crystal packing.

The short-lived antitoxin ε neutralises the toxic effects of ζ in the $\varepsilon_2\zeta_2$ complex. This is achieved by side chains of the N-terminal helix a of ε, which block the putative ATP binding pocket ζ by steric hindrance and repulsive interactions, thereby inactivating the toxin. The inactive form exists *in vivo* as long as the gene encoding ε is present and expressed in the cell cytosol.

Structural comparisons with published proteins deposited in the Protein Database (PDB) revealed significant structural homology of ζ to phosphotransferases, although the sequence homology is low (max. 15%). Despite the latter, the structural information obtained permitted determination of the catalytically important residues in ζ. Expansion of the originally defined project involved site-directed mutagenesis studies and structural analysis of the resultant mutant proteins. All mutations of the putative catalytic residues produced inactive, non-toxic ζ protein, supporting the postulation that the toxic effect of ζ is caused by a phosphotransferase activity. This is the first account of utilisation of both molecular biological and protein structural methods to describe the functional mechanism of inactivation of a TA system.