

Structural Studies of Mycobacterial Small Heat Shock Proteins.

Gemma Gargent

Abstract:

Small heat shock proteins (sHSPs) are a family of chaperones that all contain a conserved C-terminal sequence called the α -crystallin domain. Sequence alignments of the sHSPs found in mycobacterial species have revealed that the proteins are arranged into 3 classes; named *acr1*, *acr2* and *acr3*.

Previous work on the two *M. tuberculosis* sHSPs has shown that the *acr1* is a dodecamer (contrary to earlier literature reports of a nonomer) and the *acr2* is polydisperse with oligomers between 16 and 28 subunits. A negative stain EM reconstruction has shown *acr1* to be a tetrahedron with a dimer on each edge.

I have cloned and expressed a number of *acr* sHSPs from mycobacterial species that are homologous to the *acr* proteins from *M. tuberculosis*.

Nanospray Mass spectroscopy* revealed that two of these proteins, *acr2* from *M. marinum* and *M. smegmatis* formed monodisperse 24 subunit oligomers, in contrast to the *M. tuberculosis* protein. *Acr3* from *M. leprae* formed a mixture of a 16-mer and a 18mer, whereas, the *acr1* proteins from *M. marinum* and *M. smegmatis* both formed a large range of oligomers.

The *acr2* protein from *M. marinum* and *M. smegmatis* were both crystallised. The *M. marinum* *acr2* crystal was very small and did not diffract further than 10 Å whereas, the *M. smegmatis* *acr2* crystal diffracted to about 7.5 Å. From this data the space group was calculated to be I432, which suggests that the protein would have octahedral symmetry.

Finally I have taken a number of micrographs on the Technai 120 kV electron microscope of both *M. marinum* and *M. smegmatis* *acr2* at a magnification of 52,000 and a range of defocus values from 600-800nm. Preliminary analysis of the 3,165 particles picked from the S25 micrographs has revealed six-fold symmetry. As images taken using an electron microscope are projections of 3D density the six-fold axis may well be two 3-fold axes superimposed on one another; which would be compatible with the space group calculated from the crystallography dataset.

*carried out by Justin Benesch and Alex Painter (Cambridge University)