ESRF	**Experiment title:** **Structure of an esterase from *Bacillus subtilis***	**Experiment number**: LS-1532
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Report:

We are exploring the substrate specificity of an esterase from Bacillus subtilis by structural study. The protein has been characterised as a Cephalosporin C deacetylase (EC 3.1.1.41) which hydrolyses the acetyl bond at the 10-position of the antibiotic Cephalosporin C. However, we have noticed strong homology to a class of Acetyl xylan esterases (EC 3.1.1.72) which releases acetate from xylans and other xylo-oligosaccharides. Purified protein has activity on a range of substrates, including both acetylated xylan and cephalosporin C. We are curious to learn if the enzyme truly is has a broad specificity, or if it is specific for one of the substrates but with a minor activity for the other substrate.

Sequence analysis suggested that the protein is an alpha/beta hydrolase, and a catalytic triad can be deduced from sequence alignments. There is likely some novel features about this particular family of hydrolase, as analytical ultracentrifugation experiments suggest that the protein in solution is a well-defined hexamer.

Crystals were grown using the hanging drop method, mounted in a rayon loop, and flash cooled to 100K in a stream of boiling nitrogen gas. Several crystals which diffracted well on a laboratory home source were taken to the EMBL-outstation in Hamburg, and diffraction experiments were carried out on beamline X11. The crystals belong to space group R3, with strong pseudo R32 symmetry. The unit cell parameters are a=b=315.21Å, c=68.48Å (crystal form I). Data were collected to a resolution of 1.9Å. In order to obtain phases, a Se-methionine form of the protein was expressed. Crystals were obtained, but turn out to belong to space group R32, with unit cell dimensions of a=b=156.74Å, c=132.66Å (crystal form II). In spite of the apparent relation between the unit cells of these two crystal forms, crystal form I cannot be reindexed into crystal form II.

A three wavelength MAD experiment was carried out on beamline BM14 of the ESRF. Data were collected to 2.6Å starting from a spindle position suggested by the program STRATEGY (Ravelli *et al.*, 1997) and

processed using the HKL2000 suite of programs (Otwinowski & Minor, 1997). The selenium sites were determined using the program Shake&Bake at the ESRF. Phasing was done using the program MLPHARE from the CCP4 suite of programs, and phase improvement techniques applied as implemented in the program DM (Cowtan & Main, 1996). The resulting map was readily interpretable, and an initial model was built using the module XFIT in QUANTA (MSI inc.). After some refinement using REFMAC (Murshudov *et al.*, 1997), the partially refined model was used to solve the structure of crystal form II by molecular replacement using the programs AmoRe and MOLREP. Both programs determined the position of six protein molecules in the asymmetric unit. Automated refinement using Warp/REFMAC resulted in essentially complete models for eight molecules in the asymmetric unit. Six of those form a non-crystallographic hexamer, while the remaining two are one dimer in a hexamer around a crystallographic threefold axis. Further refinement is currently under way. It is expected that the two hexamers in the crystal are of sufficiently different dimensions to explain the fact that molecular replacement only located six molecules.

In addition to solving the native structure, ES complexes of an inactive mutant protein with a variety of substrates will be attempted.

References

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