ESRF	**Experiment title:** Structural Studies of Key Enzymes in Peptidoglycan Biosynthesis: Diaminopimelate epimerase DapF from *Haemophilus influenzae*	**Experiment number:** Ls1070/ls1071
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Report:

The peptidoglycan layer of bacterial cell walls is a macromolecule of unusual chemical structure, whose biosynthesis is performed by an identified pathway of enzymes. Defects or disruption of the cell wall leads to cell lysis and death due to the high internal osmotic pressure of the cell. Therefore peptidoglycan biosynthesis is an essential pathway for all bacteria. This biosynthetic pathway provides a wide range of enzyme mechanisms for study, which include non-ribosomal peptide bond synthesis and the utilisation of D-amino acids, which do not occur in proteins. Several clinically important bacteria such as the enterococci have acquired resistance to glycopeptide antibiotics such as vancomycin through the production of peptidoglycan cell wall precursors with different structural components. This is primarily due to the action of D-amino acid ligase enzymes with altered substrate specificity to that exhibited by the ligases of *Escherichia coli* and other vancomycin susceptible bacteria.

The peptidogylcan unit consists of alternating NAG-NAM polysaccharide units to which a pentapetide is attached through N-acetyl muramic acid. The sequence of the pentapeptide is unusual in that it contains a number of D-amino acids including D-alanine at the penultimate position which crosslinks with the third amino acid in an adjacent chain. In Gram-positive bacteria this third residue is usually L-lysine were as in Gram-negative species this residue is meso-diaminopimelate (M-DAP). M-DAP is an intermediate on the biosynthetic route to L-Lysine and is formed from tetrahydrodipcolinic acid via a series of enzymatic steps in bacteria and higher plants. Mammals lack this metabolic route and have to acquire L-lysine from their diet and as a result, the lysine biosynthetic pathway enzymes offer the potential for novel antibiotic targets without inhibiting mammalian homologues. M-DAP is formed from LL-DAP by the action of DAP epimerase without the aid of cofactors or metal ions in an analogous manner to proline , glutamate and aspartate racemaces . The reaction is stereospecific as the DD-DAP isomer is not a substrate for the enzyme.

Several groups have produced a variety of different DAP epimerase inhibitors (Abbot et al 1994, Gerhart et al 1990) with varying degrees of success.

We are interested in a number enzymes from the biosynthetic pathway, which perform crucial roles and are attractive as novel antibiotic targets. These include diaminopimelate epimerase (DapF) which is a PLP independent amino acid racemace involved in lysine biosynthesis and produces DL (meso) DAP from the LL isomer. Since DAP epimerase is also found in higher plants for lysine biosynthesis but is not found in animals it presents an attractive target for antibacterial and herbicide action. In Gram-negative strains of bacteria meso-DAP is incorporated into the central position of the peptidoglycan pentapeptide. We have determined the structure of diaminopimelate epimerase from *Haemophilus influenzae* at 1.75Å resolution and also from *Escherichia coli* at 2.5Å resolution using the recently published 2.5 Å structure of the *H.influenzae* enzyme (Cirilli *et al*). The native structure of the enzyme is trapped in an inactive form, as two cysteines which are known to form part of the active site, are covalently linked in a disulphide bond. We are currently determining the structure of a number of active site mutants and potential enzyme-inhibitor complexes to fully interpret the enzyme mechanism and the domain movements that occur upon substrate binding.

Although data collected at ESRF and SRS Daresbury as yet to yield an enzyme-inhibitor complex structure, we have been able to deduce that some very specific domain movements are associated with substrate/inhibitor binding. We are using a combination of molecular biological and crystallisation techniques to produce an enzyme-inhibitor complex that will enable us to identify the exact residues involved in substrate binding and catalysis.

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