

A12 PENICILLIN AND CEPHALOSPORIN BIOSYNTHESIS

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The penicillin and cephalosporin antibiotics are amongst the most important therapeutic agents in use today. These antibiotics are produced from fermentation derived materials as their chemical synthesis is not commercially viable. Unconventional steps in their biosynthesis are catalysed by mononuclear ferrous enzymes. **Isopenicillin N synthase (IPNS)** utilises iron and molecular oxygen to remove four hydrogen atoms from a linear tripeptide precursor (*L*-δ-(α-aminoacyl)-*L*-cysteinyl-*D*-valine, ACV), and thereby synthesises the labile and strained ring structure of penicillins in a single event. There is no synthetic precedent for this process. **Deacetoxycephalosporin C synthase (DAOCS)** catalyses the expansion of the penicillin nucleus into the nucleus of cephalosporins. This again is difficult chemistry.

We have obtained crystal structures for IPNS (Roach et al., 1995, *Nature*, 375, 700-704; Roach et al., 1997, *Nature*, 387, 827-830 - work done in Oxford), and very recently, we succeeded in obtaining crystal structures for DAOCS, the key cephalosporin biosynthetic enzyme (Valegård et al., *Nature*, in press - work done in Uppsala). Both DAOCS and IPNS use dioxygen-derived ferryl intermediates in catalysis, but in contrast to IPNS, the ferryl form of DAOCS is produced by the oxidative splitting of a cosubstrate, 2-oxoglutarate. An important role of the protein in these reactions is to provide a shelter to reactive intermediates, and the structures give hints how this may be achieved.

Our results together with sequence data show that members of the DAOCS-IPNS subfamily have similar three-dimensional structures. Mononuclear ferrous enzymes participate in a far broader range of reactions than their well characterised counterparts, the haem enzymes. The diversity in catalytic specificities of these enzymes on very similar structural platforms suggests that altering substrate and product specificities should be possible in the laboratory. This could be used for the synthesis of new antibiotics in order to combat the growing threat of resistant micro-organisms.

A13 Mechanistic diversity of β-lactamases.

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Many bacteria owe their survival to β-lactamases. These enzymes hydrolyse the amide bond of the β-lactam ring and, in consequence protect the producing cells against the lethal effects of the most widely utilised antibiotics: penicillins, cephalosporins and carbapenems. Two major families of β-lactamases have been identified: active-site serine and metallo-enzymes. The catalytic pathway of the former involves the formation of an acylenzyme intermediate, which is usually rapidly hydrolysed. Their 3-D structures exhibit striking similarities with those of the penicillin-sensitive DD peptidases, which are essential enzymes in the biosynthesis of the bacterial cell wall peptidoglycan and whose active-site serine is also acylated by β-lactam compounds. However, in this case, the covalent adduct is stable and the enzyme is irreversibly inactivated. The substrate profiles of active-site serine β-lactamases are very diverse and selective pressure continuously induces the emergence of enzymes with new properties, which result either from strongly altered primary structures or from a very limited number of point mutations. Metallo β-lactamases contain one or two Zn ions in their active sites. Only the most tightly bound Zn appears to be essential to the activity and stability of the proteins, but the occupancy of the second site specifically alters their catalytic properties. All known metallo β-lactamases efficiently hydrolyse carbapenems, a class of antibiotics which escape the activity of most active-site serine β-lactamases. Many β-lactamase-encoding genes are plasmid or transposon-borne and can rapidly spread among pathogenic strains, causing increasingly worrying clinical problems.

A14 Protein antibiotics and their inhibition

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Hemmings^{1,2} & Geoffrey R. Moore². Schools of Biological¹ and Chemical² Sciences, University of East Anglia, Norwich, U.K. In their bid for survival during times of nutrient or environmental stress, bacteria frequently release bacteriocins (toxins) in order to reduce competition from other microbial populations. The best known are the colicins, a large and varied family of protein antibiotics that have a characteristic domain architecture composed of a central outer-membrane receptor binding domain, an N-terminal translocation domain and a C-terminal cytotoxic domain. We have been investigating the mode of action of the 60 kDa E group colicins which bind the vitamin B₁₂ receptor, in combination with the porin OmpF, and then commandeer the periplasmic Tol protein system to intoxicate *E. coli* cells. Of the different cytotoxic activities that colicins can display, we have focused on the DNase family (ColE2 & ColE7-ColE9) which have to penetrate the inner membrane to reach their target, the bacterial genome. The talk will summarise our current understanding of how these folded proteins are able to traverse bacterial membranes and will focus on recent work from our laboratory on the affinity purification of the B₁₂ receptor, use of the yeast two-hybrid system to investigate protein-protein interactions during toxin translocation, enzymological characterisation of the 15 kDa E9 DNase domain and identification of active site residues by random mutagenesis. Colicin producing bacteria protect themselves by co-synthesising a 10 kDa immunity protein that binds and inactivates the DNase domain with a K_d of 0.1 fM. We have solved the crystal structure of this 25 kDa complex, as well as the NMR solution structures of several immunity proteins, which together define the unique mechanism by which immunity proteins inactivate colicins. In combination with biophysical analysis, protein engineering and phage display we have also uncovered how immunity proteins distinguish between closely related colicins, through a mechanism we have termed 'dual recognition'. We speculate that this protein-protein interaction mechanism may underpin specificity in many different biological contexts.

B1 The Maternal, Fetal and Postnatal Somatotrophic axes in intra-uterine Growth Retardation.

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