LPS inhibitors: key to overcoming multidrug-resistant bacteria?

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Researchers have discovered a potent inhibitor of an important enzyme in the lipopolysaccharide (LPS) pathway in gram-negative bacteria, which they hope will provide a novel treatment to overcome multidrug-resistant bacteria.

As bacterial multidrug resistance becomes more prevalent, scientists are investing an enormous amount of effort into trying to discover which of the 3,000–4,000 genes in the 'average' bacterial genome are likely to be the most useful new targets for antibiotics. Many pathways are yet to be exploited; for example, no inhibitor of any enzyme in the LPS pathway has yet been developed into a clinically useful drug. Researchers at the University of Michigan (Ann Arbor, MI, USA) have now discovered a potent inhibitor of 3-deoxy-D-*manno*-octulosonic acid 8-phosphate synthase (KDO 8-P synthase), an important enzyme in this pathway, analogues of which could prove useful against gram-negative bacteria.

KDO 8-P synthase

KDO 8-P synthase was first fully characterized in the 1970s by Paul Ray and colleagues at Burroughs Wellcome. The enzyme catalyses the condensation of phosphoenolpyruvate (PEP) and arabinose-5-phosphate (A5P) to form the monosaccharide, KDO 8-P, and phosphate. KDO 8-P is a precursor of KDO and links the membrane-bound lipid component of LPS to the rest of the core polysaccharide.

Ronald Woodard and colleagues (College of Pharmacy, University of Michigan) screened a library of 150,000 compounds obtained from Parke-Davis (now Pfizer Global) against KDO 8-P synthase[1]. The first step in the three-step screen was a fast continuous phosphate assay. Approximately 150 compounds that showed some activity in this assay were then screened in a colorimetric assay, followed by a continuous assay, in which the disappearance of the double bond in PEP using UV spectroscopy was monitored. One compound, PD404182 (Fig. 1), was discovered to be an extremely potent inhibitor. It has a K_i value of ~26 nM, which is at least four orders of magnitude lower than those of previously published inhibitors of this enzyme[1].

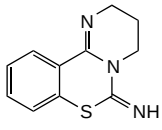

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Figure 1. The chemical structure of the KDO 8-P synthase inhibitor, PD404182.

Mechanism of action of PD404182

The likely mechanism of PD404182 is not immediately apparent from its structure. It is a relatively hydrophobic compound with few hydrogen bonding groups, and has no structural similarity to the substrates or products of KDO 8-P synthase.

In collaboration with Professor Domenico Gatti (Wayne State University, Detroit, MI, USA), Woodard and colleagues have solved the crystal structure of KDO 8-P synthase from *Aquifex aeolicus*, a hyperthermophile found in hydrothermal vaults[2]. 'Hyperthermophiles are not pathogens, but they can be good models for pathogenic bacteria,' Woodard explains. 'This enzyme is only active at high temperatures, and we found we could introduce both substrates into its active site without inducing the reaction.' They discovered that two loops of the protein close over the active site once both substrates have bound, isolating the active site from the solvent[2]. This conformational change is likely to be the rate-limiting step. Attempts to co-crystallize KDO 8-P synthase from any species of bacteria with PD404182 have not yet proven successful, although Matthew Birck, a graduate student working with Woodard, has been able to 'dock' a model of the inhibitor into the enzyme structure (Fig. 2).

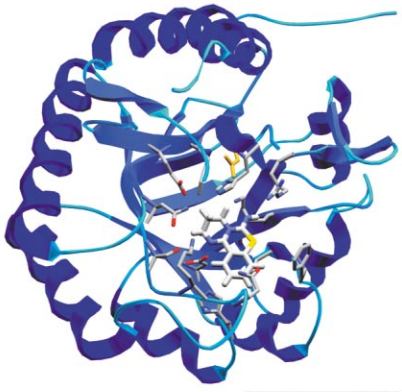

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Figure 2. Model of PD404182 docked into the active site of *E. coli* KDO 8-P synthase. [Figure kindly provided by Matthew Birck, University of Michigan (MI, USA).]

During these studies, Woodard and colleagues observed that *A. aquifex* KDO 8-P synthase, unlike its counterpart in *Escherichia coli*, requires a divalent metal ion for activity. Birck and Woodard therefore performed a phylogenetic analysis of 25 KDO 8-P synthase protein

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sequences. The resulting tree, generated using a maximum likelihood method, divided the enzymes into two clear groups with the *E. coli* and *A. aquifex* (such as *Helicobacter pylori*) sequences in different classes[3]. When other enzymes were characterized, those that were grouped with *A. aeolicus* were found to require metal for activity, whereas those (like *Salmonella typhi*) that were grouped with *E. coli*, were not. There was no other common feature between the enzymes in either class[3]. Furthermore, PD404182 was discovered to be a potent inhibitor of all enzymes in both classes apart from the hyperthermophiles. It is therefore hoped that analogues of this compound could prove to be active against a wide range of gram-negative bacteria.

Future prospects

Robert Kretsinger (University of Virginia, VA, USA) says 'Specific KDO 8-P synthase inhibitors could well be viable drugs, but it is important to remember that only one in a hundred promising drugs ever reaches the market.' Woodard agrees with this cautious assessment: 'Our inhibitor does not kill bacterial cells as well as we would like.' However, it will be difficult to improve its binding through chemical modification until a crystal structure of the enzyme–inhibitor complex has been solved. Woodard's group is initially concentrating on trying to improve the solubility of PD404182 and its ability to cross the cell wall but, so far, they have not had much success.

However, if these problems can be overcome, PD404182 would have two important advantages as a lead compound. First, it has previously been patented as a non-steroidal anti-inflammatory drug, so some data on its toxicity and metabolic properties is available in the literature. Also, compounds targeting the LPS pathway could be useful as drugs even if they are not cytotoxic. The lipopolysaccharide layer prevents many antibiotics that are active against gram-positive bacteria from entering gram-negative cells. It is possible that a compound like PD404182, given as adjuvant therapy with one of these antibiotics, could break down this layer enough to allow the antibiotic entry into the cells.

References

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2 Duewel, H.S. *et al.* (2001) Substrate and metal complexes of 3-deoxy-D-*manno*-octulosonate-8-phosphate synthase from *Aquifex aeolicus* at 1.9A resolution. *J. Biol. Chem.* 276, 8393–8402

3 Birck, M.R. and Woodard, R.W. (2001) *Aquifex aeolicus* 3-deoxy-D-*manno*-2-octulosonic acid 8-phosphate synthase: a new class of KDO 8-P synthase. *J. Mol. Evol.* 52, 205–214

A sticky end for pathogens?

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Novel flow-based assay technologies designed to study the interaction between pathogens and host cells could lead directly to new drug discovery targets. LigoCyte Pharmaceuticals (Bozeman, MT, USA) has developed two promising anti-infective technology platforms: a Proteoflow™ system to develop new targets for anti-infective therapeutics and vaccines, and nanoparticle technology that can be used to provoke an immune response without the risk of infection.

ProteoFlow™ assays

The flow-based assays originally developed by LigoCyte were inspired by the cell–cell interactions involved in inflammation, where white blood cells are recruited and transported by shear forces in the blood and are required to stop instantaneously at the site of inflammation. This ability to attach to inflamed tissue has been subsequently studied using a flow-based assay consisting of a glass tube lined with endothelial cells through which blood components are flowed, and observing and quantifying the characteristics of the fluorescently labelled white blood cells by video microscopy. By altering the expression of proteins, such as receptors, on the cells and adding various purified proteins, antibodies or inhibitors, the interactions of the immune cells with the endothelial cells can be characterized, and compounds that inhibit inflammation can be studied (Fig. 1). Similar models using epithelial cells simulate the lining of the intestines and airways.

LigoCyte has now applied this technology to the development of assays to study the interaction of pathogens such as *Escherichia coli* and *Candida albicans,* both with host cells and homotypic binding between the pathogens themselves. For example, *E. coli* form colonies by adhering to other *E. coli* cells that have already attached to mucosal epithelium, which results in the upregulation of virulence factors. Inhibition of the initial attachment to the host cell would, therefore, reduce the likelihood of colonization and further infection at existing sites of inflammation, such as a surgical

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