Crystal structure of the laminin-binding protein Lbp of Streptococcus pyogenes

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The crystal structure of the laminin-binding protein Lbp (Spy2007) of Streptococcus pyogenes has been determined to 2.45 Å resolution. According to recent studies, Lbp mediates adhesion of the major human pathogens S. pyogenes and S. agalactiae to the human basal lamina glycoprotein laminin. It has been shown to be essential in vitro models of adhesion and invasion. In this study, Lbp from S. pyogenes strain M1 was cloned, expressed in E. coli as a (His)6-tagged protein and purified by IMAC. Recombinant Lbp yielded crystals belonging to the monoclinic space group P21, and of good diffraction quality. The structure of Lbp to a resolution of 2.45 Å was solved by molecular replacement using an ensemble of search models from homologous proteins and was refined to an Rcryst of 18.6% and Rfree of 24.7%. The structure consists of a long helical backbone connecting two lobes which enclose a cobalt ion in a characteristic histidine/glutamate metal binding site. It is largely similar to that of homologous proteins, which are implicated in metal transport, but is among the first bacterial laminin-binding proteins to be determined.

The crystal structure of Lbp will allow further investigations into the molecular basis of laminin-binding by human pathogens and give new insight into host-pathogen interactions. As Lbp is immunogenic and conserved in all S. pyogenes strains, its structure may guide development of an efficient vaccine.

Keywords: structural biology of bacterial pathogenesis, adhesion, metalloprotein structures

Structural studies of the glucuronate-xylulose pathway implicated in diabetic complications

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Diabetes mellitus is one of the world’s foremost health concerns. Much of the pathology caused by the disease is due to the complications of sustained hyperglycemia. The seven-step glucuronate-xylulose (GX) pathway is over activated in diabetics. GX activity results in the depletion of D-chiro-inositol, a molecule pivotal in insulin signalling, and also of the protective osmolyte, myo-inositol. This pathway is only partially biochemically and structurally characterized. The three-dimensional structure of myo inositol oxygenase (MIOX), the enzyme that catalyses the first committed step in the GX pathway, has recently been determined at the University of Auckland, New Zealand. Inhibition of this pathway is an attractive target for the development of a novel class of therapeutics to treat diabetic complications. Our aims are to extend this investigation by (1) studying human MIOX with inhibitors and as part of a complex with the second member of the pathway, D-glucuronate reductase (aldehyde reductase-1), and by (2) investigating the three uncharacterized members of the GX pathway; L-gulonate dehydrogenase, dehydro-L-gulonate carboxylase and xylulokinase. An overview of the project and current progress will be presented.

Keywords: diabetes, inositol, glucuronate-xylulose pathway

Structural studies of isopropylmalate synthase from Mycobacterium tuberculosis.

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Although tuberculosis (TB) is a curable disease under present treatments, it is still a global threat largely due to the vast number of people infected with Mycobacterium tuberculosis in developing countries. Furthermore, the global impact of TB is widening due to the increasing prevalence of multi-drug resistant strains. New drugs, particularly ones that operate by alternative pathways from current regimens, are vital. The leucine biosynthetic pathway is essential for M. tuberculosis survival, and is not found in humans, making it an attractive target for the design of new anti-TB drugs. The three-dimensional structure of isopropylmalate synthase (IPMS), which catalyses the first committed step in this pathway, has been determined at high resolution atomic detail by X-ray crystallography using multiwavelength anomalous dispersion methods. A range of additional structures has been obtained by co-crystallisation or by soaking crystals with additives. These structures include complexes with: native substrate (ketoisovalerate); product analogue (citrate); competitive inhibitor (3-bromopyruvate); and feedback inhibitor (leucine). This repertoire of IPMS structures, together with the unliganded form, can be complemented with functional, mutagenic, and bioinformatic analyses to provide a general mechanism for activity and a sound template for the design of inhibitors specifically targeting IPMS.

Keywords: branched-chain amino acid biosynthesis, aldol-condensation reaction, active-site structure

New polymorphs of Pigment Red 53:2

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Pigment Red 53:2 is an industrial organic lake red azo pigment. It is synthesised by diazotation of 2-amino-5-chloro-4-methylbenzenesulfonic acid with subsequent coupling on β-naphthol and final treatment with CaCl2 [1]. The pigment precipitates as a nanocrystalline powder, which is hardly soluble in water and all solvents. An extensive polymorph screening was performed using different synthetic methods and various re-crystallisation procedures. The new polymorphs show orange, red and brown