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Structural studies of fimbrial proteins of the oral bacteria *Actinomyces oris*

Karina Persson, Rolf Claesson and Nicklas Strömberg, *Departament of Odontology, Umeå University, Sweden.* E-mail: karina.persson@odont.umu.se

Dental plaque is one of the most complex biofilms known and consists of hundreds of bacterial species. The development of dental plaque is initiated by the attachment of salivary proteins to the tooth surface, followed by adherence of early colonizing bacteria, such as the Gram-positive *Actinomyces oris*. These bacteria form the initial biofilm that is used as an attachment surface for late colonizers, many of them Gram-negatives. Crucial factors for these microorganisms to be able to network with other bacteria and cells are their surface adhesins and fimbriae.

A. oris expresses two forms of fimbriae, FimP and FimA. With FimP the bacteria can bind to salivary proline-rich proteins attached to the tooth surface and with FimA to carbohydrate structures on the surface of bacteria and host cells. The two types of fimbriae are encoded by two separate operons. Each operon encodes a putative adhesin, followed by a shaft protein that is polymerized into the fimbrial stalk, and finally a sortase, a cysteine transpeptidase that is responsible for the polymerization. The sortase cleaves the shaft protein between a threonine and a glycine in an LPLTG motif in the C-terminus. Next, a lysine side chain in the central part of another shaft protein forms an amide linkage to the C-terminal threonine. To gain more insight into oral biofilm formation and to the structure of Gram-positive fimbriae we are studying the these fimbrial proteins using X-ray crystallography.

To date we have solved the crystal structures of the FimP shaft protein to 1.9 Å resolution as well as its sortase to 2.4 Å. The sortase consist of an eight stranded β-barrel with a flexible lid protecting the active site. The lid is held in a closed conformation by the coordination of a calcium ion [1]. The *A. oris* FimP fimbria is extremely stable, which is a requirement for survival in the oral cavity. The stability can be explained by the crystal structure of the shaft protein. The protein is elongated and comprised by three IgG-like domains, stabilized by two disulfide bonds, two covalent isopeptide bonds and one tightly bound calcium ion.

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Structural basis of ligand and pathogen recognition by the collectins

Annette Shrive,[a] Amy Shaw,[a] Abdul Ahmed,[a] Jenny Paterson,[a] Carrie Smallcombe,[a] Ken Reid,[b] Mary Deadman,[c] Derek Hood,[c] Uffe Holmskov,[d] Howard Clark,[e] Jens Madsen,[e] Trevor Greenhough,[a] [a]*Institute of Science & Technology in Medicine, School of Life Sciences, Keele University, (UK).* [b]*MRC Immunochemistry Unit, University of Oxford, (UK).* [c]*John Radcliffe Hospital, University of Oxford, (UK).* [d]*Institute of Molecular Medicine, University of Southern Denmark, (Denmark).* [e]*Dept of Medicine, University of Southampton, (UK).* E-mail: a.k.shrive@keele.ac.uk

The biological activity of collectins is exerted through Ca-dependent binding of cell surface carbohydrate, including lipopolysaccharides and phospholipids, peptidoglycans and glycosaminoglycans. The residues in the carbohydrate-binding pocket that coordinate to both the calcium ion and the ligand are highly conserved. Variability in other binding determinants in the binding pocket is, however, evident throughout the family.

Our high resolution structures of a biologically and therapeutically active fragment of hSP-D [1,2] provide an understanding of the ligand specificity of the collectins. New structural work on the interaction of lipopolysaccharide fragments of the lung pathogen *Haemophilus influenzae* with this recombinant hSP-D fragment reveals a novel mode of interaction and sheds light on variation in bacterial strain recognition. Efficient recognition appears to require multiple binding interactions in the SP-D binding pocket suggesting a general mode of LPS binding by hSP-D based on conserved elements of the LPS core.

Further recent structural work has led to the structure determination of the collectins conglutinin and CL-46. Conglutinin is known to bind Ca-dependently to iC3b, a component of complement protein C3 deposited on cell surfaces. Structural studies of ligand binding to conglutinin, in particular with its favoured ligand GlcNAc, show a unique binding specificity that has not previously been observed in any collectin structure.

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Crystal Structure of *Staphylococcus aureus* MGT in complex with a lipid ii analog

Chia-Ying Huang,[a,c] Hao-wei Shih,[a,d] Li-Ying Lin,[a] Wei-Chieh Cheng,[a] Chi-Huey Wong,[a] Che Ma[a]. [a]*Genomics Research Center, Academia Sinica, 128 Academia Road, Section 2, Taipei 115, Taiwan; Institutes of* [b]*Biochemistry and Molecular Biology and* [c]*Microbiology and Immunology, National Yang-Ming University, 155 Linong Street, Section 2, Taipei 112, (Taiwan), and* [d]*Department of Chemistry, National Taiwan University, 1 Roosevelt Road, Section 4, Taipei 106, (Taiwan).* E-mail: ying@gate.sinica.edu.tw.

Penicillin binding proteins (PBPs), the final enzymes to assemble cell wall, are the key enzymes for bacterial cell wall synthesis and good candidates for design of new antibiotics [1]. Recently infections from β-lactam-resistant *Staphylococcus aureus* or vancomycin resistant *Entercoccus faecium* become a serious medical problems[2]. Moenomycin is the only potential inhibitor for the transglycosylase reaction, and between moenomycin and transglycosylase has been defined the interaction from the high quality crystal structure of transglycosylase in complex with Moenomycin [*Escherichia coli* PBP1b (PDB 3FWL) [3], *Aquifex aeolicus* PGT (PDB 3D3H)[4], *S. aureus* PBP2 (PDB 2OLV)[5] and *S. aureus* MGT (PDB 3HZS)[6]]; however, the substrate Lipid II has never been observed from crystal structure before. In this study, several Lipid II analogs are designed to co-crystallize with the *S. aureus* MGT, and the complex structure of *S. aureus* MGT-Lipid II analog is determined at 2.3Å resolution. The molecular interaction between Lipid II and transglycosylase provides basis for understanding the mechanism of glycan chain elongation and structure-based antibiotic design.

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