MS36.P06

Acta Cryst. (2011) A**67,** C472

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.

[1] K. Persson, *Acta Crystallographica Sect D-Biological Crystallography* **2011***, 67*, 212-217.

Keywords: adhesin, isopeptide bond, biofilm

MS36.P07

Acta Cryst. (2011) A**67,** C472

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.

[1] A.K. Shrive, H.A. Tharia, P. Strong, U. Kishore, I. Burns, P.J. Rizkallah, K.B.M. Reid, T.J. Greenhough, *Journal of Molecular Biology* **2003**, *331*, 509-523. [2] A.K. Shrive, C. Martin, I. Burns, J.M. Paterson, J.D. Martin, J.P. Townsend, P. Waters, H.W. Clark, U Kishore, K.B.M. Reid, T.J. Greenhough *Journal of Molecular Biology* **2009,** *394*, 776-788.

Keywords: lectin, immune system, pathogen recognition

MS36.P08

Acta Cryst. (2011) A**67,** C472-C473

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.

[1] C. Goffin, J.M. Ghuysen, *Microbiol Mol Biol Rev* **1998**, *62*, 1079-1093. [2]G. Taubes, *Science* **2008**, *321*, 356-361. [3]M.-T. Sung, Y.-T. Lai, C.-Y. Huang, L.-Y. Chou, H.-W. Shih, W.-C. Cheng, C.-H. Wong, C. Ma, *Proceedings of the*

National Academy of Sciences **2009**, *106*, 8824-8829. [4]Y. Yuan, S. Fuse, B. Ostash, P. Sliz, D. Kahne, S. Walker, *ACS Chem Biol* **2008**, *3*, 429-436. [5]A.L. Lovering, L.H. de Castro, D. Lim, N.C.J. Strynadka, *Science* **2007**, *315*, 1402-1405. [6] H. Heaslet, B. Shaw, A. Mistry, A.A. Miller, in *Journal of Structural Biology*, **2009**, *167*, 129-135.

Keywords: lipid II, moenomycin, transglycosylase

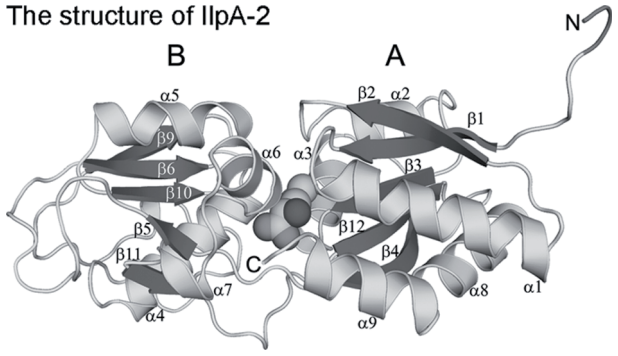
MS36.P09

Acta Cryst. (2011) A**67,** C473

Structural analysis of Toll-like receptor 2-activating lipoprotein

Sangheon Yu,[a] Na Yeon Lee,[b] Soon-Jung Park,[b] Sangkee Rhee,[a] [a]*Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, (Korea).* [b]*Department of Environmental Medical Biology and Institute of Tropical Medicine, Yonsei University College of Medicine, Seoul 120-752, (Korea).* E-mail: shyu@snu.ac.kr

IlpA, a surface protein of the human pathogen *Vibrio vulnificus*, is the first lipoprotein to be characterized in *Vibrio* spp. as a major immunostimulant. Previously, it was characterized that IlpA was subject to lipidation at its N-terminal cysteine residue. The resulting IlpA then activates Toll-like receptor 2 in human cells, and induces overproduction of proinflammatory cytokines closely associated with septic shock in infected individuals. To identify structural features of IlpA, we determined the crystal structure of IlpA at 2.6 Å resolution. Specifically, IlpA consists of two homologous domains, each with α/β topology, similar to the structure of substrate-binding protein which is a component of ATP-binding cassette transporter. In fact, binding of L-methionine was observed in the pocket between the two domains, suggesting that IlpA is an L-methionine-binding protein. The structural features of IlpA in this study, along with the immunological properties of IlpA identified previously and other substrate-binding proteins, suggest that substrate-binding lipoproteins of ATP-binding cassette transporter present at the bacterial cell surface could serve as pathogen-associated molecular patterns to Toll-like receptor 2, causing host immune responses against infection.



[1] S. Yu, N.Y. Lee, S.J. Park, S. Rhee, *Proteins* **2011,** *79*, 1020-1025.

Keywords: lipoprotein, structure, substrate-binding

MS36.P10

Acta Cryst. (2011) A**67,** C473

Structure of the catalytic domain of *H. pylori* cholesterol-α-glucosyltransferase

Sang Jae Lee,[a] Byung Il Lee,[b] Hye-Jin Yoon,[a] Se Won Suh,[a,c] [a]*Department of Chemistry, College of Natural Sciences, Seoul National University (Korea).* [b]*Cancer Cell and Molecular Biology Branch, Division of Cancer Biology, Research Institute, National Cancer Center (Korea).* [c]*Department of Biophysics and Chemical Biology, College of Natural Sciences, Seoul National University (Korea).* E-mail: sanzelee@gmail.com

α-Glucosyl cholesterol and its derivatives are the major cell wall components of *Helicobacter pylori*, also playing an important role in immune evasion and survival. *H. pylori* makes α-glucosyl cholesterol by glucosylating cholesterol extracted from the plasma membranes of human gastric mucosa cells, using the enzyme cholesterol-α-glucosyltransferase. Here we present the crystal structure of the catalytic domain of cholesterol-α-glucosyltransferase from *H. pylori* at 1.50 Å resolution, providing a platform for discovering specific inhibitors of *H. pylori* cholesterol-α-glucosyltransferase that could be developed as novel antibiotics. This work was funded by Korea Ministry of Education, Science, and Technology, National Research Foundation of Korea; Basic Science Outstanding Scholars Program, World-Class University Program, and Innovative Drug Research Center for Metabolic and Inflammatory Disease; Korea Ministry of Health, Welfare & Family Affairs (Korea Healthcare Technology R&D Project, Grant no. A092006).

Keywords: cholesterol-α-glucosyltransferase, HP0421, helicobacter pylori

MS36.P11

Acta Cryst. (2011) A**67,** C473

Structure of the *Streptococcus pyogenes* β-NAD+ glycohydrolase-inhibitor complex

Ji Young Yoon,[a] Jieun Kim,[b] Se Won Suh,[a,b] [a]*Department of Chemistry, College of Natural Sciences, Seoul National University (Korea).* [b]*Department of Biophysics and Chemical Biology, College of Natural Sciences, Seoul National University (Korea).* E-mail: bandij7@snu.ac.kr

Streptococcus pyogenes (group A streptococcus; GAS) secretes several extracellular proteins which contribute to pathogenesis. Among them, β-NAD^+ glycohydrolase (SPN) is an important virulence factor. The mechanism for pathogenesis is intracellular β-NAD^+ depletion within the host cell due to the β-NAD^+ hydrolytic activity of SPN. SPN is also toxic to the bacterium itself; therefore, GAS encodes the *ifs* gene, whose product (IFS) is an endogenous inhibitor of the NAD^+ glycohydrolase. In order to understand the inhibition mechanism of SPN by IFS, we have determined the crystal structure of the SPN-IFS complex at 1.8 Å resolution. SPN is an atypical member of the ADP-ribosyltransferase superfamily, lacking a canonical binding site for the protein substrate. The SPN-IFS complex is stabilized by numerous hydrogen bonds and electrostatic interactions. In the complex structure, IFS covers the active site of SPN and blocks the binding of β-NAD^+.

Keywords: β-NAD+ glycohydrolase, IFS, streptococcus pyogenes

MS36.P12

Acta Cryst. (2011) A**67,** C473-C474

Crystal structures of Eis proteins from *M. tuberculosis* and *M. smegmatis*

Kyoung Hoon Kim,[a] Doo Ri An,[b] Se Won Suh,[a,b] [a]*Department of Chemistry, College of Natural Sciences, Seoul National University (Korea).* [b]*Department of Biophysics and Chemical Biology, College*