MS5-P24 Structural studies of AggC, an novel O-GlcNAc transferase involved in protection of virulence-associated cell proteins in *Staphylococcus aureus*

Chia-I Liu[1], Tzu-Ping Ko[2], Wen-Yih Jeng[3,4], Wei-Jung Chang[2,3], Jhih-Tong Ge[1], Andrew H.-J. Wang[2,3,5]

1. School of Medical Laboratory Science and Biotechnology, College of Medical Science and Technology, Taipei Medical University, Taipei 10031, Taiwan
2. Institute of Biological Chemistry, Academia Sinica, Taipei 11529, Taiwan
3. Core Facilities for Protein Structural Analysis, Academia Sinica, Taipei 11529, Taiwan
4. University Center for Bioscience and Biotechnology, National Cheng Kung University, Tainan 70101, Taiwan
5. Graduate Institute of Translational Medicine, College of Medical Science and Technology, Taipei Medical University, Taipei 10031, Taiwan

email: ponpiqq@gmail.com

Glycosylation of bacterial cell wall proteins play a critical role in bacterial pathophysiology. Two novel O-linked glycosyltransferases (OGTs), AggB and AggC, decorate all SD (serine-aspartate) repeats of adhesins with N-acetylglucosamine (GlcNAc) moieties, which containing the virulence factors ClfA (a fibrinogen-binding clumping factor A), ClfB, SdrC (SD repeats C), SdrD, SdrE of *S. aureus* and SdrF, SdrG SdrH of *S. epidermidis.* Recently, it has been demonstrated that glycosylated SD repeats proteins can facilitate bacterial adhesion, immune evasion, colonization, persistence and invasion of host tissue. This specific modification also promotes *S. aureus* replication in the bloodstream of mammalian hosts. AggB and AggC modify all SD repeats proteins by an ordered mechanism, with AggC appending the sugar residues proximal to the target SD repeats, followed by additional modification by AggB. Here we report two crystal structures of *S. aureus* AggC, as a binary complex with citrate (2.8 Å) and as a ternary complex with UDP and GlcNAc (2.2 Å). The structures provide clues to the enzyme catalytic mechanism, implying how AggC recognizes target peptide sequences, and reveal the fold of the unique β-meander domain and a core catalytic domain with GT-B fold. Structure-based mutagenesis of AggC was also performed to explore the roles of amino acids involved in substrate binding. In summary, this information will accelerate the rational design of biological experiments to investigate AggC functions and also help the design of inhibitors as a therapeutic target.

Keywords: glycosyltransferase, adhesion, Staphylococcus aureus

MS5-P25 Crystal structure of a novel *Caulobacter crescentus* oxidoreductase and its complexes

Helena Taberman[1], Martina Andberg[2], Anu Koivula[2], Juha Rouvinen[1], Tarja Parkkinen[1]

1. Department of Chemistry, University of Eastern Finland, P.O. Box 111, 80101 Joensuu, Finland
2. VTT Technical Research Centre of Finland Ltd, P.O. Box 1000, 02044 VTT, Finland

email: helena.taberman@uef.fi

Using biocatalysts in producing chemicals from renewable raw materials is an emerging industrial sector and a scope of active research. Further development in this field is required to help fuel the next generation of biorefineries and contribute to the bioeconomy. We have been interested to find novel D-xylose converting enzymes for the exploitation of hemicellulose containing biomasses. Some D-xylose dehydrogenases belong to the Gfo/Idh/MocA enzyme family, including the glucose-fructose oxidoreductase (GFOR) of *Zymomonas mobilis*. During studies on D-xylose dehydrogenases, we encountered an open reading frame (ORF) from fresh water bacterium *Caulobacter crescentus* automatically annotated as a GFOR or as a D-xylose dehydrogenase. To evaluate whether this ORF is involved in D-xylose conversion, it was cloned, expressed and purified from *Saccharomyces cerevisiae*.

The *C. crescentus* oxidoreductase has a high sequence identity (49%) with the *Zm* GFOR (2, PDB ID: 1H6A). *Zm* GFOR enzyme uses a tightly bound $NADP^+$ cofactor, which is regenerated in the oxidation/reduction cycle, presumably through a ping-pong reaction mechanism (1). The main substrates of GFOR are D-glucose and D-fructose, the former oxidized to D-gluconolactone whereas the latter is reduced to D-sorbitol. The characterisation of the *C. crescentus* oxidoreductase demonstrated that it can catalyse both the oxidation and reduction of several different saccharides to corresponding aldonolactones and alditols, respectively.

In this study, the crystal structures of the holo-form of *Cc* oxidoreductase and its complexes with different sugars and sugar polyols have been determined. The structures demonstrate a two-domain structure composed of the classical N-terminal Rossmann fold for dinucleotide binding (3) and of a β-sheet formed of seven, mostly antiparallel, β-strands, separated by three α-helices. Two *Cc* oxidoreductase monomers form a dimer by packing their open-faced β-sheets together. The structures are currently under refinement. The results provide insight into the cofactor and ligand binding, and help us to elucidate the exact reaction mechanisms of the enzyme.

The work was part of the Finnish Centre of Excellence in White Biotechnology-Green Chemistry (Academy of