

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

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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

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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.

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References

1. Cleland, W. W. (1963) *Biochem. Biophys. Acta* **67**, 104-137.
2. Nurizzo, D. et al. (2001) *Biochemistry* **40**, 13857-13867.
3. Rao, S. T. & Rossmann, M. G. (1973) *J. Mol. Biol.* **76**, 241-156.

Keywords: oxidation/reduction cycle, biomass, crystal structure, enzyme mechanism

MS5-P26 The *cis*-peptide conformation at loop I of the Hypoxanthine phosphoribosyltransferases is not essential for the binding of substrates

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Hypoxanthine phosphoribosyltransferases (HPRT; EC 2.4.2.8) catalyze the transfer of a phosphoribosyl group from 5-phospho-a-D-ribosyl-1-pyrophosphate (PRPP) to a purine base (hypoxanthine, guanine or xanthine) to form pyrophosphate (PP_i) and a purine nucleotide, inosine monophosphate (IMP), guanosine monophosphate (GMP), or xanthosine monophosphate (XMP), respectively. At the active site one of the loops involved in the binding of the substrates (loop I) all the HPRT structures known up to now have a peptide bond in a *cis* conformation allowing the binding of one phosphate group. We have solved the structure of the HPRT from *Chromobacterium violaceum* in the presence of the substrates PRPP, IMP and GMP; and in all of these structures we have not observed the presence of the *cis* conformation at loop I. This protein presents a glycine at the second position of this three residue loop, while the usual amino acid present at this position are lysine or arginine that are involved in the ability or inability to carry out the pyrophosphorolysis (reverse) reaction, respectively.

Keywords: HPRT, Loop I, *cis*-peptide conformation