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 (PDB ID: 1H6A)[1]. *Zm* GFOR enzyme uses a tightly bound NADP+ cofactor, which is regenerated in the oxidation/reduction cycle, presumably through a ping-pong reaction mechanism.[2] The main substrates of GFOR are D-glucose and D-fructose, the former oxidized to D-gluconolactone hereas 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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