

Ferredoxin-NADP⁺ reductase (FNR) catalyzes the redox reaction between NAD(P)⁺ and NAD(P)H with the electron carrier protein, Ferredoxin (Fd). Recently, new FNR subfamily that shares a structural homology to NADPH-dependent thioredoxin reductase (TrxR) was identified. We have solved the first crystal structure of the TrxR-like FNR from the green sulfur bacterium *Chlorobaculum teidium* and reported the several unique structural features of TrxR-like FNR [1]. The additional C-terminal sub-domain, that covers the *re*-face of the isoalloxazine ring of FAD, was newly found in *C. teidium* FNR. The unique asymmetric domain arrangement suggests the bending motion of the hinge region between the FAD and NADPH binding domains. Then, the crystal structure of *Bacillus subtilis* FNR, classified into TrxR-like FNR, was reported as a complex with NADP⁺ [2]. On the basis of amino acid sequence analysis, it has been recently reported that photosynthetic purple bacterium *Rhodospseudomonas palustris* also has a TrxR-like FNR. In this study, we have examined the crystal structure of *Rps. palustris* FNR by X-ray crystallography in order to confirm the reported structural features of TrxR-like FNR.

The crystal structure of *Rps. palustris* FNR was determined by the molecular replacement method at 2.4 Å resolution. The C-terminal sub-domain containing the FAD stacking Tyr residue was confirmed in the *Rps. palustris* FNR structure. *Rps. palustris* FNR exists as a homodimer in the crystallographic asymmetric unit. When the FAD domain of one protomer is superimposed on that of the other, one NAD(P)H domain is rotated by 16.5° with respect to the other. The domain arrangements of *Rps. palustris* FNR is more open, when compared to those of *C. tepidum* FNR and *B. subtilis* FNR. Sequential comparison of the all NADPH domains of TrxR-like FNRs and TrxRs proposes the unique trajectory of the domain, which might be closely related to the replacement of the structurally conserved C-terminal sub-domain during the catalytic cycle.

[1] N. Muraki, D. Seo, T. Shiba, T. Sakurai, G. Kurisu, *J. Mol. Biol.* **2010**, *401*(3), 403-14. [2] H. Komori, D. Seo, T. Sakurai, Y. Higuchi, *Protein Sci.* **2010**, *19*(12), 2279-2290.

Keywords: flavoprotein, photosynthesis, reductase

MS93.P20

Acta Cryst. (2011) **A67**, C775

Hydrolysis of the thioester intermediate in UDP-glucose dehydrogenases

Patrícia Borges,^a J. Rocha,^a A. Popescu,^b D. Mil-Homens,^b I. Sá-Correia,^b A. Fialho,^b C. Frazão,^a ^a*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras (Portugal).* ^b*Institute for Biotechnology and Bioengineering, Center for Biological and Chemical Engineering, Department of Bioengineering, Instituto Superior Técnico, Universidade Técnica de Lisboa, Lisbon (Portugal).* E-mail: pborges@itqb.unl.pt

Members of the *Burkholderia cepacia* complex (BCC) are serious respiratory pathogens in immunocompromised individuals and in patients with Cystic Fibrosis. They are exceptionally resistant to many antimicrobial agents, have the capacity of spreading between patients, and lead to declining lung function with necrotising pneumonia. BCC members express often a mucoid phenotype associated with the secretion of the exopolysaccharide cepacian. There is much evidence hinting for cepacian as a virulence factor of BCC.

Uridine-5'-diphosphoglucose dehydrogenase (UGD) is responsible for the NAD-dependent two fold oxidation of UDP-glucose (UDP-Glc) to UDP-glucuronic acid (UDP-GlcA), which is a key step in cepacian biosynthesis. Mutagenic studies have been performed on the active site of UGDs and crystallographic structures have been produced in order to help the elucidation of the complex mechanism of action in

this family of sugar-nucleotide modifying enzymes. However, the catalytic residues responsible for last mechanistic step, the hydrolysis of a covalently bonded thioester intermediate, which is simultaneously the rate determining and the only irreversible step in the mechanism, have remained elusive.

The UGD from *Burkholderia cepacia* (BceC) crystal structure was determined at 1.75 Å resolution. Its superposition with human and other bacterial UGDs showed a common active site with high structural homology. The family contains a strictly conserved tyrosine residue (Y10 in BceC) within the glycine-rich motif, (GXGYXG) of its N-terminal Rossmann-like domain. Several BceC Y10 mutants were also constructed revealing only residual dehydrogenase activity, which prompted their crystal structures determination too. The crystals of native BceC and its mutations Y10S and Y10K belonged to space-group P2₁2₁2₁, showed similar cell dimensions, and contained 4 independent molecules in the asymmetric unit. Their structures were determined at 1.75, 1.70 and 2.80 Å resolution, respectively, and led to R_{work}/R_{free} of 16.3/19.7 %, 15.5/18.5 % and 22.8/26.3 %, with acceptable Ramachandran diagrams. The available information on UDP nucleotide-sugar 6-dehydrogenase family were analyzed with our kinetic and structural data on BceC and its mutants, leading to the characterization of the conserved tyrosine as a key catalytic residue in UGDs rate determining step, the final hydrolysis of the enzymatic thioester intermediate. Its localization in the vicinity of Y10 OH group allows the stabilization of the forming thiolate upon scission of the thioester bond, by direct proton transfer from the solvent water. In absence of Y10 the thioester hydrolysis may still proceed, but only when a solvent water molecule happens to be in place for donation of the stabilizing proton to the transient thiolate, as corroborated by a comparison of the experimental activation entropies between BceC and its mutation Y10F [1], [2].

[1] J. Rocha, A.O. Popescu, I. Sá-Correia, A.M. Fialho, C. Frazão *Acta Crystallogr* **2010**, *F66*, 269-271. [2] J. Rocha, A.O. Popescu, P. Borges, D. Mil-Homens, I. Sá-Correia, A.M. Fialho, Carlos Frazão *J. Bact.* **2011**, *accepted for publication*.

Keywords: dehydrogenase, exopolysaccharide, fibrosis

MS93.P21

Acta Cryst. (2011) **A67**, C775-C776

Coenzyme binding in a highly specific isocitrate dehydrogenase

Navdeep Sidhu,^a Louis T.J. Delbaere,^{b,c} George M. Sheldrick,^a ^a*Department of Structural Chemistry, University of Goettingen, Goettingen (Germany).* ^b*Department of Biochemistry, University of Saskatchewan, Saskatoon (Canada).* ^c*Deceased October 5, 2009.* E-mail: nsidhu@shelx.uni-ac.gwdg.de

Isocitrate dehydrogenase catalyzes the first oxidative and decarboxylation reactions in the citric acid cycle. It also lies at a branch point with the glyoxylate bypass, which makes it possible for some organisms to grow on acetate as the sole carbon source. The monomeric enzyme from *Corynebacterium glutamicum* is highly specific for its substrate isocitrate and coenzyme nicotinamide adenine dinucleotide phosphate (NADP), which it prefers to nicotinamide adenine dinucleotide by a factor of 50,000 [1]. Here, we present the 1.9 angstrom crystal structure of the enzyme in complex with its coenzyme and the magnesium ion cofactor (Protein Data Bank accession code 3mbc). The overall structure is similar to the previously described structures of the monomeric enzyme from *Azotobacter vinelandii* in complex with isocitrate [2] and NADP [3], and that from *C. glutamicum* in the apo form [4]. In combination with the latter, we find that coenzyme binding in the holoenzyme may be approximately described by the lock-and-key mode even though a second molecule in the asymmetric unit in