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 tepidum* 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. tepidum* 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 with NADP\(^+\) [2]. On the basis of amino acid sequence analysis, it has been recently reported that photosynthetic purple bacterium *Rhodospseudomonas palaisiris* also has a TrxR-like FNR. In this study, we have examined the crystal structure of *Rps. palaisiris* FNR by X-ray crystallography in order to confirm the reported structural features of TrxR-like FNR.

The crystal structure of *Rps. palaisiris* 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. palaisiris* FNR structure. *Rps. palaisiris* 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. palaisiris* FNR is more open, when compared to those of *C. tepidum* FNR and *B. subtilis* FNR. Sequential comparison of 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.

**Keywords:** flavoprotein, photosynthesis, reductase

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Hydrolysis of the thioster intermediate in UDP-glucose dehydrogenases

Patricia Borges,*a J. Rocha,*a A. Popescu,*b D. Mil-Homens,*b I. Sá-Correia,*a A. Fialho,*a 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 (UDG) 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 thiostere intermediate, which is simultaneously the rate determining and the only irreversible step in the mechanism, have remained elusive.

The UGD from *Burkholderia cepacia* (BccC) 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 BccC) within the glycine-rich motif, (GXXGXXG) of its N-terminal Rossmann-like domain. Several BccC Y10 mutants were also constructed revealing only residual dehydrogenase activity, which prompted their crystal structures determination too. The crystals of native BccC and its mutations Y10S and Y10K belonged to space-group P2\(_1\)2\(_1\)2\(_1\), 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 \(R_{work}/R_{ref}\) 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 BccC 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 thioster intermediate. Its localization in the vicinity of Y10 OH group allows the stabilization of the forming thiolate upon scission of the thioster bond, by direct proton transfer from the solvent water. In absence of Y10 the thioster 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 BccC and its mutation Y10F [1], [2].

**Keywords:** dehydrogenase, exopolysaccharide, fibrosis

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Coenzyme binding in a highly specific isocitrate dehydrogenase

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

Isocitrate dehydrogenase catalyzes the first oxidative and deoxybonylation 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 model even though a second molecule in the asymmetric unit in...
the present structure is in the apo form and differs significantly in
conformation with the holo form. In comparison with the A. vinelandii
holoenzyme structure, which displayed a closed conformation which is
inaccessible to icoticrate, the present structure shows the enzyme in an
open conformation that allows the substrate access to the active site.

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Delbaere, Proteins: Structure, Function, and Bioinformatics 2006, 63, 100-112.

Keywords: isocitrate dehydrogenase, citric acid cycle, coenzyme specificity

MS93.P22


TrpS – evolutionary paths to catalytic efficiency
Olga Mayans, Arnaud Basle, Rainer Bucher
Institute for Protein Research, Osaka University, 3-2 Yamada-
oka, Suita, Osaka 565-0871, (Japan). *Biozentrum, Univ Basel, (Switzerland).
E-mail: Olga.Mayans@liv.ac.uk

The efficiency of metabolic pathways in the cell largely relies on
enzyme association processes that lead to the compartmentation
of biochemical reactions. This allows to overcome barriers in metabolite
production derived from the high intracellular concentrations of
compounds (cellular crowding) that affect diffusion, equilibria,
reactions rates and side reactions. It also prevents cross-talk between
competing pathways and relieves cellular stress by maintaining high
local concentrations of compounds but low total cellular levels.
In forming metabolic compartments, multifunctional enzymes
(permanent or transient) are formed that couple sequential reactions.
Often, enzymes within a pathway not only evolve mutual recognition,
but also complex modes of communication that allow them to share
small compounds and substrates (channeling) as well as to mutually
control their activities (allostery).

Tryptophane synthase (TrpS) is a well established model system for
the study of substrate channeling and allostery in enzymes. TrpS
consists of a central beta,-dimer flanked by two alpha-subunits in a
linear a-b-a-b-a arrangement. The alpha-subunit cleaves indole-glycerol-
phosphate into glyceraldehyde-phosphate and indole, while subunit
beta condenses indole with L-serine to yield L-tryptophan using
pyridoxal phosphate (PLP) as cofactor. Catalytic efficiency is achieved by
the channelling of indole from the active site of subunit alpha to that
of beta through a hydrophobic, 25 Å long interconnecting tunnel. The
reactions of the active sites are kept in phase by switching both subunits
between open and close conformations to prevent indole loss. Further,
the subunits mutually activate each other through the transmission of
allosteric effects. Thus, the TrpS catalytic cycle requires a delicate
orchestration of structural changes in the enzyme machinery [1].
Recently, subunits alpha and beta have been found as isolated proteins
–TrpA and TrpB– in archean genomes. These variants, thought to be
evolutionary remnants, display crippled association properties and
distinct catalytic efficiencies [2]. Aiming to retrace the evolution of
the sophisticated TrpS machinery from rudimentary TrpA and TrpB
modules, we have elucidated the crystal structures of these TrpA and
TrpB variants as well as their transient complex. The models serve as
templates to analyse the role of indels (insertion and deletion sequence
motifs) as dominant mechanistic factors in the evolution of catalytic
features. We conclude that inter-subunit communication in TrpS is
the result of a “gain-of-function” in its component subunits caused by
motif deletions. Further, we uncover an alternative enzymatic strategy
by which the simpler TrpA and TrpB enzymes achieve catalytic
efficiency as high as that of TrpS, but in the absence of intricate subunit
interrelations. This work sheds light, from a structural perspective, on
the diversification of enzymatic strategies to catalytic proficiency.

Sakakibara, T. Hase, M. Kusunoki, Journal of Biological Chemistry 2006, 281,
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Distant residues of maize glutamine synthetase affecting substrate affinity
Masami Kusunoki, Takeshi Ozaki, Atsushi Nakagawa, Toshiharu Hase
Institute for Protein Research, Osaka University, 3-2 Yamada-
oka, Suita, Osaka 565-0871, (Japan). *Department of Biotechnology,
Faculty of Engineering, University of Yamanashi, 4-3-11 Takeda, Kofu
City, Yamanashi 490-8511, (Japan). E-mail:mkusunki@yamanashi.
ac.jp

Glutamine synthetase (GS, EC 6.3.1.2) is an enzyme that catalyzes
the incorporation of ammonia into glutamate to form glutamine with
concomitant hydrolysis of ATP. Plant glutamine synthetase plays a
crucial role in the assimilation and re-assimilation of ammonia derived
from a wide variety of metabolic processes during plant growth and
development.

Glutamine synthetase from eucaryotes has an oligomeric structure
consisting of 10 identical subunits to form two pentamer rings with
52 symmetry. The ten catalytic centers are located between two
adjacent subunits in each ring, and the two rings are held together by
residues around Phe150, which are distant from the catalytic centers.
Kinetic study of P146G, F150G and delF150 mutant enzymes with
crystallographic study of F150V and F150G revealed that the ring-ring
contact sites are crucial for high affinity for the substrate glutamate and
the glutamate may be incorporated into the catalytic centers through
the regions near the two ring interfaces.

Alanine-scanning mutagenesis around Phe150 and kinetic study
revealed that residues responsible for increasing $K_m$ for glutamate
are located at the regions spanning the ring-ring interfaces and the
eentry sites of glutamate into the catalytic centers. These residues are
distant from the catalytic centers and hence are likely to contribute to the
affinity of glutamate to the enzyme in addition to the affinity of
the catalytic centers themselves for glutamate. To verify this working
hypothesis crystals of two mutants G241A and W243A in these regions
were grown and analyzed at 2.55Å and 2.80 Å resolutions, respectively.
The refined crystal structures of them indicated that the structures except for the mutated residues are similar to the wild type enzyme, without significantly changing the
structure of the wild type enzymes. This work sheds light, from a structural perspective, on
the diversification of enzymatic strategies to catalytic proficiency.

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Keywords: enzyme, crystal, plant