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 tedium* 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. tedium* 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 *Rhodopseudomonas 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.

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Hydrolysis of the thioester intermediate in UDP-glucose dehydrogenases

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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, (GXG**Y**XG) 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 $P2_12_12_1$, 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_{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].

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Coenzyme binding in a highly specific isocitrate dehydrogenase

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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

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 isocitrate, the present structure shows the enzyme in an open conformation that allows the substrate access to the active site.

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TrpS – evolutionary paths to catalytic efficiency

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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 allows them to share small compounds and substrates (*channelling*) as well as to mutually control their activities (*allostery*).

Tryptophane synthase (TrpS) is a well established model system for the study of substrate channelling and allostery in enzymes. TrpS consists of a central beta$_2$-dimer flanked by two alpha-subunits in a linear a-b-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 archea 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.

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Distant residues of maize glutamine synthetase affecting substrate affinity

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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 entry 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 distant from the catalytic centers were prepared 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 catalytic centers. This study supports the structural elements separate from the catalytic centers contribute to increasing the affinity for substrate glutamate.

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