involve a proton relay mechanism via chemical exchange of hydrogen nuclei along a precise chain of water molecules.

Keywords: inorganic pyrophosphatase, neutron macromolecular crystallography, catalytic mechanism

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Structural insights into the enzyme catalysis of dissimilatory sulfite reductase

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Sulfite reductase mediates the reduction of sulfite to sulfide in sulfate-reducing bacteria. The existence of multi-forms of enzymes for the sulfite reduction and the details of the catalytic mechanism that can lead to a distribution of three products— trithionate ($S_3O_6^{2-}$), thiosulfate ($S_2O_3^{2-}$) and sulfide (S^{2-}) had been a subject of puzzle and controversy. The crystal structures of two active forms of dissimilatory sulfite reductase (Dsr) from *Desulfovibrio gigas*, Dsr-I and Dsr-II, are compared at 1.76 and 2.05 Å resolution, respectively [1]. The dimeric $\alpha_2\beta_2\gamma_2$ structure of Dsr-I contains eight [4Fe-4S] clusters, two saddle-shaped sirohemes and two flat sirohydrochlorins. In Dsr-II, the [4Fe-4S] cluster associated with the siroheme in Dsr-I is replaced by a [3Fe-4S] cluster. This structural feature allows Thrβ145 to position itself closer to the [3Fe-4S] in Dsr-II to replace the role of the Cysβ188 that ligates the [4Fe-4S] in Dsr-I. Electron paramagnetic resonance (EPR) of the active Dsr-I and Dsr-II confirm the co-factor structures, whereas EPR of a third but inactive form, Dsr-III, suggests that the siroheme has been demetallated in addition to its associated [4Fe-4S] cluster replaced by a [3Fe-4S] center. In Dsr-I and Dsr-II, the sirohydrochlorin is located in a putative substrate channel connected to the siroheme and capped by a dynamic loop from the ferredoxin domain. Cysβ198, which is located between the two types of porphyrins, exhibits double conformations. The γ-subunit C-terminus is inserted into a positively charged channel formed between the α- and β-subunits, with its conserved terminal Cysγ104 side chain covalently linked to the CHA atom of the siroheme in Dsr-I. In Dsr-II, the thioether bond is broken, and the Cysγ104 side chain moves closer to the bound sulfite at the siroheme pocket. Moreover, the γ-subunit C-terminus reveals another arrangement with an interaction between Cysγ93 and Cysγ104 in both Dsr forms. A second sulfite, interacting with the conserved Lysγ100, has been identified, implicating this site as the entry into a second putative substrate channel. These different forms of Dsr offer structural insights into a mechanism of sulfite reduction that can lead to $S_3O_6^{2-}$, $S_2O_3^{2-}$ and S^{2-} [1].

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Structural insights into function of heterodimeric prenyltransferase

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Over 50,000 structurally diverse isoprenoids, which are built from C_5 isoprene units, are widely distributed in nature. Many kinds of isoprenoids, such as vitamins, hemes, and membrane lipids, are essential components of the cellular machinery of all organisms. Prenyltransferases, the so-called prenyl diphosphate synthases, catalyze consecutive head-to-tail condensations of isopentenyl diphosphates (IPP, C_5) on an allylic-substrate, such as dimethylallyl diphosphate (DMAPP, C_5) or farnesyl diphosphate (FPP, C_{15}), to form linear prenyl diphosphates with various chain-lengths. The linear prenyl diphosphates are common precursors of the carbon skeletons for all isoprenoids. According to the geometry of the newly formed double bonds of the products, prenyltransferases can be divided into two major classes, *trans*- and *cis*-prenyltransferases. Furthermore, the *trans*-prenyltransferases can be divided into two sub-classes, homo- and heterooligomeric enzymes. Compared to the homooligomeric enzymes, the structure-function relationships of heterooligomeric enzymes are not really investigated. The heterooligomeric enzymes are comprised of two different subunits, large and small subunits. The large subunits have been considered to participate in the substrate binding and the condensation reactions because the subunits possess the catalytic motifs conserved in the homooligomeric enzymes. In contrast, the small subunits do not possess such motifs and show very low amino-acid sequence identities with the homooligomeric enzymes. Thus, the function of the small subunits has not yet been clearly understood. In order to elucidate the molecular mechanism of heterooligomeric *trans*-prenyltransferases, particularly with respect to the role of the small subunits, we have determined the crystal structures of heterodimeric hexaprenyl diphosphate (HexPP, C_{30}) synthase from *Micrococcus luteus* B-P 26 (*Ml*-HexPPs) both in the substrate-free form and in complex with 3-desmethyl FPP (3-DesMe-FPP), an analogue of FPP [1].

The structure of the large subunit HexB is composed of mostly antiparallel a-helices joined by connecting loops. Despite the very low amino-acid sequence identity and the distinct polypeptide chain-lengths between the small subunit HexA and HexB, the structure of HexA is quite similar to that of HexB. Two aspartate-rich motifs and the other characteristic motifs in HexB are located around the diphosphate part of 3-DesMe-FPP. The aliphatic-tail of 3-DesMe-FPP is accommodated in a large hydrophobic cleft starting from the two substrate-binding sites of HexB and penetrating to the inside of HexA. Residues in both subunits participate in forming the wall of this cleft. These structural features suggest that the large subunit HexB catalyzes the condensation reactions and the small subunit HexA is directly involved in the product chain-length control in cooperation with HexB.

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Keywords: enzyme, isoprenoids, protein crystallography

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Crystal structure of ferredoxin-NAD(P)$^+$ reductase from *Rhodopseudomonas palustris*

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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