nicotinamide head of $NADP^+$, as well as allowing the formation of a putative substrate binding pocket [2].

These three structures have been instrumental in increasing our understanding of the mechanism, from a structural perspective, of the BVMOs. The critical structure of the substrate-bound form, however, has thus far remained elusive. As a result, a number of questions remain. These include how the enzyme can accommodate both $NADP^+$ and the substrate simultaneously, how the substrate specificity is determined, and which residues play key roles in stabilizing the various intermediates. As such, the substrate-bound structure is necessary to properly guide attempts to engineer these enzymes towards specific substrates.

We have solved a crystal structure of CHMO showing the first substrate-bound, catalytic conformation of any classical BVMO. This structure provides insight into the intricate movements that are integral to its reaction mechanism, involving the enzyme, its cofactors, and its substrate. Of note, a unique conformation of the $NADP^+$ cofactor creates an environment that allows for the formation of an active site pocket in close proximity to the flavin ring system. This allows the positioning of the substrate into a catalytically relevant location. A number of residues are implicated in stabilizing this conformation and maintaining the substrate in the active site until catalysis is completed.

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Keywords: oxygenase, mechanism, flavoenzyme

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Structural and functional studies of pseudomonas mesoacidophila MX-45 trehalulose synthase and trehalulose hydrolase

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Various diseases related to the over-consumption of sugar make a growing need for sugar substitutes. Sucrose is an inexpensive and readily available d-glucose donor, thus industrial potential for enzymatic synthesis of the sucrose isomers trehalulose and/or isomaltulose from sucrose is large. The naturally occurring structural isomer of sucrose, trehalulose is produced by sucrose isomerases. Two adjacent gene homologs, *mutA* and *mutB* from *Pseudomonas mesoacidophila* MX-45 have been characterized and demonstrated an activity on these sugars.

The enzyme MutA hydrolyzes the substrates trehalulose, isomaltulose, and sucrose into glucose and fructose, with a highest activity on trehalulose, whereas the enzyme MutB is a trehalulose synthase and catalyses the isomerisation of sucrose to mainly trehalulose. Since these genes are responsible for uptake and utilization of trehalulose and isomaltulose, sucrose or the reaction products may be involved in transcriptional regulation [1].

Mature MutB and MutA proteins displayed 52% sequence identity. These two enzymes belong to family GH13 as classified in the CAZy database.

We have recently cloned, purified, crystallized and solved the crystal structures of MutB and MutA.

Three-dimensional crystal structures, native- and mutant complexes of the trehalulose synthase MutB reveal an aromatic clamp playing an essential role in substrate recognition and in controlling the reaction specificity [2] and highlight essential residues for binding the glucosyl- and fructosyl-moieties [3]. These structures allowed defining the mode of action of this enzyme, providing important information on the recognition mode of sugars as well as different stages of the catalytic reaction mechanism [4].

Comparative studies highlighting the structural differences between trehalulose synthase and isomaltulose synthase, gave important information on the structural determinants responsible for the specificity of products formed by the sucrose isomerases. To complete and validate the information currently known, several mutants of the enzyme MutB are currently being studied. The new mutations affect the affinity of the enzyme towards its substrate and to change the specificity of formed products by native enzyme MutB. MutA is the first enzyme described that is able to hydrolyze trehalulose, and therefore might be useful for some industrial processes, e.g., treatment of sticky cotton fiber. The precise description of the reaction mechanism therefore should give valuable insights into the functioning of this enzyme.

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Keywords: sucrose isomerase, crystal structure, trehalulose

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X-ray and neutron crystallographic structure-based mechanism of archaeal inorganic pyrophophatase from *Thermococcus thioreducins*

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Soluble inorganic pyrophosphatase from *Thermococcus thioreducins* (Tt-IPPase) has been crystallized in five unique metal or substrate complexes within monoclinic and rhombohedral space groups. As a result, the X-ray crystallographic structures of IPPase were determined revealing a 1) substrate-free Tt-IPPase with Ca^{+2} analyzed at room temperature (22°C) at 1.65Å (PDB ID 3R5V); 2) Ca^{+2}-bound IPPase complexed with the $P_2O_7^{-4}$ substrate at 1.44Å (PDB ID 3Q4W); 3) Ca^{+2}-bound IPPase complexed with the $P_2O_7^{-4}$ substrate at 1.35Å (PDB ID 3Q9M); 4) Mg^{+2} activated IPPase bound to hydrolyzed substrate at 0.99Å (PDB ID 3Q46); and 5) Mg^{+2}-bound IPPase complexed with SO_4^{-2} at 1.08Å (PDB ID 3Q5V). In addition we have determined the neutron crystallographic structure of substrate-free bound to Ca^{+2} at 2.50Å analyzed at room temperature (PDB ID 3Q3L). Based on these structural data, we were able to visualize and hypothesize the sequence of catalytic events associated with the hydrolysis of pyrophosphate substrate for the first time in an archaea system. We report a catalytic mechanism that involves an Asp in the active site serving as a principal acceptor for the deprotonation of a water molecule that bridges two metals. As a result, the formation of an attacking nucleophile OH^- toward the hydrolysis of pyrophosphate substrate is proposed. In addition the deprotonation of water may also

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

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