

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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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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X-ray and neutron crystallographic structure-based mechanism of archaeal inorganic pyrophosphatase 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 Ca²⁺ analyzed at room temperature (22°C) at 1.65Å (PDB ID 3R5V); 2) Ca²⁺-bound IPPase complexed with the P₂O₇⁴⁻ substrate at 1.44Å (PDB ID 3Q4W); 3) Ca²⁺-bound IPPase complexed with the P₂O₇⁴⁻ substrate at 1.35Å (PDB ID 3Q9M); 4) Mg²⁺ activated IPPase bound to hydrolyzed substrate at 0.99Å (PDB ID 3Q46); and 5) Mg²⁺-bound IPPase complexed with SO₄²⁻ at 1.08Å (PDB ID 3Q5V). In addition we have determined the neutron crystallographic structure of substrate-free bound to Ca²⁺ 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