

RATIONALIZATION OF INHIBITOR BINDING IN SERINE/THREONINE PHOSPHATASES

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Serine/Threonine Protein Phosphatases are important in many cellular processes including protein synthesis, signal transduction glycogen metabolism in muscle and immunosuppression. Many prokaryotic organisms produce phosphatase inhibitors and can be toxic to humans if ingested. The surface of Ser/Thr Phosphatases contain an inhibitor-binding loop which is independent of the catalytic mechanism of the enzyme, but appears to be important in inhibitor activity. How this loop determines inhibitor-specificity between the phosphatases is unknown. There is speculation that the loop may move to accommodate different inhibitors, giving each phosphatase its own response to specific inhibitors. We have solved the structures of Protein Phosphatase-1 (PP1) bound to two different natural product inhibitors, okadaic acid and microcystin-LA(2H). Both of these inhibitors bind in a similar manner to the phosphatase, exhibiting analogous interactions with the inhibitor-binding loop. Importantly, the inhibitor-binding loop is in almost identical positions in both structures. A similar structure using a mutant PP1 with the inhibitor-binding loop from calcineurin substituted in reveals repositioning of only specific amino acid side-chains, but identical positioning of the backbone of the loop. These results indicate that inhibitor specificity in Ser/Thr Phosphatases is most likely only due to specific inhibitor-enzyme interactions within the inhibitor-binding loop and not structural rearrangements of the loop.

Keywords: PROTEIN PHOSPHATASE, INHIBITORS, ENZYME REGULATION

ON THE ENZYMATIC MECHANISM OF LUMAZINE SYNTHASE

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Lumazine synthase catalyzes the penultimate step in the biosynthesis of riboflavin. The monocyclic 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione (1) condensates with 3,4-dihydroxy-2-butanone-4-phosphate (2) to the bicyclic 6,7-dimethyl-8-ribityllumazine (3). In the following step catalyzed by riboflavin synthase compound 3 is converted to riboflavin. Many organisms lack an uptake system for riboflavin and are therefore dependent on its endogenous synthesis. Moreover, neither lumazine synthase or riboflavin synthase are present in mammals. Both are therefore attractive targets for antimicrobial therapy. We have recently solved the structure of lumazine synthase from *Saccharomyces cerevisiae* in form of a complex with an inhibitor and from *Aquifex aeolicus* in its native form. In the present study we have determined complexes of both enzymes with several inhibitors and also the native structure of the yeast enzyme. The inhibitors are constructed such that they mimic the structure of compound (1), a putative intermediate and compound (3). Most contacts between the inhibitors are well preserved throughout all complexes. His88 in *A. aeolicus* (*S. cerevisiae*: His97) and Phe22 in *A. aeolicus* (*S. cerevisiae*: Trp26) assume different well-defined conformations depending on the presence or absence of an inhibitor. Mutants of *B. subtilis* lumazine synthase were constructed to test the role of several residues in the conversion. We propose a detailed mechanism, which involves several protonation and deprotonation steps assisted by His88 (*A. aeolicus*) / His97 (*S. cerevisiae*). Lys135 in *A. aeolicus* (*S. cerevisiae*: His148) might have a specific role in the reorientation of a phosphorylated intermediate.

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CRYSTAL STRUCTURE OF YEAST PHOSPHOFRUCTOKINASE

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Extensive crystallographic studies have been carried out on bacterial Phosphofructokinase1 but until now, no eukaryotic PFK1 structure has been solved. It is an allosteric key enzyme in glycolysis that catalyses the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate in the presence of Mg-ATP. In baker's yeast PFK1 is a hetero- octamer (composed of 4 α and 4 β subunits) of molecular weight approximately 800 kDa. As a result of early gene duplication in eukaryotes, each subunit consists of two homologous repeats. The redundant parts evolved to develop new functionalities, allowing eukaryotic PFK in general to become more responsive to an even larger range of allosteric effectors than the bacterial enzyme. In yeast a second gene duplication event is supposed to have occurred, forming two different types of subunits (α and β) with heterogeneous terminal regions responsible for subunit association. As a result of these duplication events, one yeast PFK1 octamer consists of 16 prokaryotic monomer-like units.

The size of yeast PFK1 makes the crystallography a challenge but we recently solved its structure in complex with fructose-6-phosphate by X-ray crystallography. Native diffraction data have been collected to 2.9 Angstrom under cryogenic conditions. Phase information was obtained by two successive molecular replacement steps using the biological *E. coli* PFK tetramer as search model, as well as electron microscopy data and internal symmetry information. Quality of the electron density map was dramatically improved by NCS averaging, leading to a map that could be analyzed unequivocally. The crystal structure of the baker's yeast PFK1 thus obtained will be presented and compared with its bacterial homologue.

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THE STRUCTURE OF AMYLOSUCRASE IN COMPLEX WITH FRUCTOSE

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Amylosucrase from *Niesseria polysaccharea* catalyses the transfer of the glucosyl moiety from sucrose onto a α -1-4 glucan. This enzyme belongs to the family 13 of glycoside hydrolases. Within this family, a common reaction mechanism involving a glucosyl-enzyme intermediate is believed to operate. For efficient transglucosylation, hydrolysis of the covalent intermediate must be minimized. In amylosucrase the active site is placed in the bottom of a deep bifurcated pocket (1,2). When a polymer is bound, the active site is inaccessible to water (3), and the covalent intermediate would be protected from hydrolysis. It is not known when the other reaction product leaves the active site, but if this happens before transglucosylation the intermediate would be exposed to solvent. Temporary storage of fructose seems to be an option that has to be considered.

Here we present the structure of amylosucrase mutant E328Q in complex with fructose, refined to a resolution of 2.2 Å. The fructose is bound in its open form inside a small pocket in close proximity of the active site, a feature that has not previously been observed in other enzymes from this family.

References

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