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THE STRUCTURE OF X-PDAP FROM *LACT. LACTIS* GIVES NEW PERSPECTIVES FOR STRUGGLE AGAINST PATHOGENS

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PepX, X-prolyl dipeptidyl aminopeptidase (X-PDAP) from *Lactococcus lactis*, is a dimeric enzyme catalyzing with a high specificity the removal of Xaa-Pro dipeptides from the N-termini of peptides. Its crystal structure was solved at 2.3 Å resolution using the multiple isomorphous replacement method. PepX presents an uncommon complex fold compared with other aminopeptidases. Each monomer consists of 763 amino acids and comprises four distinct domains. The serine active site lies in the central and larger domain that has an α-β-hydrolase fold. Curiously, the specificity pocket is mainly built from residues of a small helical domain that is also, with the N-terminus moiety, essential for dimerization. This observation reveals the close relation between dimerization and specificity. On the C-terminus extremity the enzyme is completed with a striking voluminous domain supposed to act as a tropic factor toward lipidic membrane. The very long loop projecting out of this domain can be involved in the access of the substrate to the core of the enzyme. The data provided here give new insights to further explore the role of PepXs from beneficial micro-organisms as well as pathogenic ones. On the basis of homologies with X-PDAP from *Streptococci*, specific inhibitors designed against structural features of PepX could efficiently eliminate or at least slow down the growth of pathogenic *Streptococci* without disrupting the normal metabolism of the host.

Keywords: α-β HYDROLASE FOLD AMINOPEPTIDASE PEPX

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STRUCTURE AND INACTIVATION OF TRIOSEPHOSPHATE ISOMERASE FROM *ENTAMOEBA HISTOLYTICA*

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In most species, triosephosphate isomerase (TIM) is a homodimer in which each monomer is folded into a (β/α)8 barrel. This enzyme is essential for the net production of ATP in glycolisis. It is considered, therefore, as a promising target for the design of drugs aimed at selectively modifying non-conserved amino acids present in the enzyme from several human pathogens. We have determined the high-resolution structure of *Entamoeba histolytica* TIM (EhTIM), which is the first crystallographic structure of a protein from this pathogen, and characterized the effect of methylmethane thiosulfonate (MMTS) on the activity and conformational properties of the enzyme.
Purified EhTIM was crystallized by the vapor diffusion technique. Native data to 1.5 Å resolution was collected at SSRL beamline 9-1. The structure was determined by molecular replacement using TbTIM (Trypanosoma brucei brucei TIM) as a search probe. The final structure consists of 260 residues for each monomer, and 692 water molecules. The EhTIM dimer is not symmetric, differences between monomers were observed in the backbone and side chains of residues belonging to the interface loop 3.
EhTIM is completely inactivated by MMTS, and thereby dissociated into stable and compact monomers that possess considerable secondary structure. This is the first example of a monomeric state of TIM produced by chemical modification. Two factors that may contribute to the unusual stability of the derivatized monomer are proposed: Conformational rearranges at the interface after dissociation; and intramonomeric contacts formed by the inserted residues.
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CRYSTALLIZATION AND STRUCTURAL STUDIES OF TYPE I CHLORAMPHENICOL ACETYLTRANSFERASE

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Chloramphenicol acetyltransferase (CAT) is responsible for bacterial resistance to chloramphenicol. It catalyzes inactivation of the antibiotic by acetyl group transfer from acetyl coa to one or both hydroxyl groups of chloramphenicol. Three different classes of cat have been found in gram negative bacteria. All natural cat variants are homotrimers. The crystal structure of Type III Cat has been determined at 1.75 Å resolution (leslie, 1990). Type I Cat which is encoded by the transposon Tn9 possesses some unique properties which are not observed in other cat variants. Besides chloramphenicol, it is capable of inactivating also fusidic acid, a steroidal inhibitor of bacterial protein synthesis acting at the elongation phase Type I Cat cloned in a pbr322 is constitutively expressed in *e.coli* under the p1 promoter was purified and crystals with a resolution limit of 2.22 Å; have been obtained. The crystals have the symmetry of space group *p*1 and the unit cell dimensions a = 96.46, b = 113.86, c = 114.21 Å α = 119.9˚, β = 94.1˚ and γ = 98.6˚. These dimensions are consistent with four to six trimers per unit cell corresponding to a solvent fraction ranging from 65% to 47%. Refinement of the purification scheme led to a more rational and consistent crystallization procedure, which yields data-quality crystals with a wide range of precipitating agents. Furthermore, more convenient crystal forms with a reduced number of molecules per asymmetric unit have been obtained. We pursue structure determination of the apoenzyme (will be presented in the conference) as well as structure of then enzyme with the substrate and its inhibitors

Keywords: **CHLORAMPHENICOL CHLORAMPHENICOL ACETYLTRANSFERASE TYPE I FUSIDIC ACID**

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STRUCTURAL FLEXIBILITY AND ALLOSTERIC TRANSITION: THE CASE OF GLUCOSAMINE-6-PHOSPHATE DEAMINASE

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Glucosamine-6-phosphate deaminase is an allosteric enzyme that catalyzes the reversible reaction of glucosamine 6-phosphate and a water molecule to form fructose 6-phosphate and ammonia. Seven new crystallographic structures of the R conformer of the enzyme with different ligands (of the active site, the allosteric sites or both; native and mutants), and seven new crystallographic structures of the T conformer (native and mutants), coupled to previously reported structures of the T and R conformers, generates a detailed description of the heterotropic allosteric transition in which structural flexibility is shown to play a central role. In the particular case of the T structure, seven alternate conformations for the active-site lid and three for the C-terminus, were refined using isotropic B-factors. Some of these conformers approach in geometry that of the R conformer. Furthermore, the direction of the atomic vibrations obtained with anisotropic B refinement supports the hypothesis of an oscillating rather than a tense T state. Most of the allosteric enzymes present kinetics behaviors in concordance with concerted transition models. This property has been difficult to explain in structural terms. The T state presenting an oscillation which approach to the R geometry describe the process of the ligand binding to the R conformer as the capture of the R geometry approached during the oscillating T state . We founded that this oscillating behavior is part of the allosteric transition process in other allosteric enzymes.
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Keywords: **ALLOSTERISM, FLEXIBILITY, ENZYME**