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STRUCTURAL STUDIES OF *DROSOPHILA MELANOGASTER* dUTPase

O. Barabas[1, 2] Zs. Dubrovay[1] V. Harmat[2] J. Kovari[1] E. Takacs[1] I. Zagyva[1] G. Naray-Szabo[2] B. G. Vertessy[1]
[1]Biological Research Center, Hungarian Academy of Sciences Institute of Enzymology Karolina Ut 29 BUDAPEST H-1113 HUNGARY [2]Protein Crystallography Laboratory, Department of Theoretical Chemistry, Lorand Eotvos University, P.O. Box 32, H-1518, Budapest, Hungary

Deoxyuridine triphosphate nucleotidohydrolases (dUTPases) play a unique preventive role in DNA repair by excluding uracil from DNA. Lack of dUTPase causes chromosome fragmentation and thymine-less cell death. The enzyme is therefore a promising target for both basic biomedical research and multifarious disease control. Quaternary organization of the enzyme is also unique: the homotrimer has three active sites, each built of sequence motifs from all the three subunits. Developmental regulation of the enzyme from *Drosophila melanogaster* is suggested to be involved in thymine-less apoptosis. Fruit fly enzyme is the first eukaryotic dUTPase being characterized in functional details. Functional studies show significant differences between the previously characterized prokaryotic dUTPases and this enzyme, such as altered conformational flexibility of the active site and unique structural effect of the Mg^{2+}-cofactor. It is an adequate model to study evolution of enzyme mechanism. The enzyme is a stable homotrimer in solution, as shown by analytical gel filtration and differential scanning microcalorimetry.
Single crystals of the enzyme in complex with substrate analogous nucleotide ligands have been grown. Analysis of a data set, collected at LURE, shows that crystals belong to the space group *P*4122, the asymmetric unit contains a monomer, and the contact regions expected to form intermolecular contacts are solvent exposed. For the first time, a monomeric form of dUTPase can be investigated in the crystal phase. Detailed examination of the monomer conformation and the grounds of its stability under the crystallization conditions offer important clues for understanding protein folding.

Keywords: dUTPase DNA REPAIR ALLOSTERISM

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A NOVEL COMBINATION OF TWO CLASSIC CATALYTIC SCHEMES

A. Shaw [1] R. Bott[1] C. Vonhreim[2] G. Bricogne[1] A. G. Day[1] S. Power[1]
[1]Genencor International Inc. 925 Page Mill Rd. PALO ALTO CA 94109 USA
[2]Global Phasing Ltd., Sheraton House, Castle Park, Cambridge CB3 0AX, UK

The crystal structure of an alkaline *Bacillus* cellulase catalytic core, from glucoside hydrolase family 5 enzyme, reveals a novel combination of the catalytic machinery of two classic textbook enzymes. The enzyme has the expected two glutamate residues in close proximity in the active-site of a retaining cellulase, that are unexpectedly combined with a serine-histidine-glutamate catalytic triad. Structure and sequence analysis of the glucoside hydrolase family 5 reveal that the triad is conserved, and variations of it occur. The purpose of this novel catalytic triad appears to be to control the protonation of the acid/base glutamate, facilitating the first step of the catalytic reaction, protonation of the substrate, by the proton donor glutamate.

Keywords: **CELLULASE CATALYTIC TRIAD ENZYMOLOGY**

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CRYSTALLOGRAPHIC STUDIES OF 12-OXOPHYTODIENOATE REDUCTASES FROM TOMATO

C. Breithaupt[1] P. Macheroux[2] A. Schaller[2] R. Huber[1] T. Clausen[1]
[1]Max-Planck Institut Fuer Biochemie Abteilung Strukturforschung Am Klopferspitz 18a MARTINSRIED 82152 GERMANY [2]Eidgenoessische Technische Hochschule, Institut fuer Pflanzenwissenschaften, Universitaetsstr. 2, CH-8092 Zuerich, Switzerland

Being constantly attacked by various pathogens and herbivores plants have developed a complex system to defend themselves against prospective colonists. The plant hormone jasmonic acid (JA), well known as plant growth regulator, and structurally related cyclic oxylipins that resemble the animal prostaglandins have been shown to act as signal molecules in the defense cascade. A key step in the biosynthesis of JA and its derivatives is the reduction of 12-oxophytodienoate (OPDA) by the FMN-dependent OPDA-reductases (OPRs). Several OPR isozymes exist in tomatoes, that differ in their subcellular localisation and their stereoselectivity to the chiral OPDA. We have determined the structure of the cytosolic OPR1 to 1.9 Å resolution by molecular replacement using yeast Old Yellow Enzyme as model. Part of the active site is composed of a hydrophobic tunnel, formed to fit the preferred stereoisomer 9R,13R-OPDA. In the native crystals a completely defined PEG400 molecule binds into the tunnel thus mimicking the substrate and stabilizing the structure by numerous hydrophobic interactions, which explains the absolute need of PEG400 for crystallization. PEG400 could be exchanged by the substrate after several soaking steps. In contrast to the monomeric OPR1, the peroxisomal OPR3 that reduces the JA-precursor 9S,13S-OPDA is dimeric in the crystal as seen in the recently solved structure. Dimerization directly affects the acitve site architecture, which might be of importance for the regulation of OPR3 *in vivo*.

Keywords: OPR, FLAVOENZYME, PLANT DEFENSE

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CRYSTAL STRUCTURE OF TRYPANOSOMAL TRANS-SIALIDASE, A KEY ENZYME IN PARASITE PATHOGENESIS

A. Buschiazzo[1] M.F. Amaya[1] M.L. Cremona[2] A.C. Frasch[2] P.M. Alzari[1]
[1]Institut Pasteur Structural Biology And Chemistry 25, Rue Du Dr. Roux PARIS 75015 FRANCE [2]Biotechnological Research Institute, UNSAM San Martin, Buenos Aires ARGENTINA

The intracellular parasite *Trypanosoma cruzi*, etiologic agent of Chagas' disease, sheds a developmentally regulated surface trans-sialidase (TS) which is involved in key aspects of parasite-host cell interactions. Although it is homologous to viral and bacterial sialidases, the *T. cruzi* enzyme behaves as a highly efficient sialyl-transferase not dependent on a sialyl-nucleotide donor substrate. Here we present the three-dimensional crystal structure of trans-sialidase at 1.6 Å resolution, alone and in complex with lactose and sialic acid derivatives. TS crystallyzed in space-groups *P*1 or $P2_12_12$, only after mutating seven surface-located residues. A comparative analysis of the different TS structures, together with the structure of TrSA, the sialidase from *T. rangeli* (which albeit being 70% identical, performs a strict hydrolytic activity), let us extract functional information. The new position of the conserved Trp312 permits the simultaneous binding of lactose as the sialic-acid acceptor substrate. Tyr342 (a catalytic residue) and Tyr119 (which replaces a Ser in TrSA), show a flexible conformation, its positions moving drastically when sialic acid binds to the catalytic pocket. These tyrosines play fundamental roles in the catalyzed transfer reaction, as confirmed by mutagenesis experiments. We propose that TS has a distinct binding site for the sialic acid-acceptor carbohydrate, implying a sequential catalytic mechanism, at difference with the ping-pong mechanism typical of sialidases. Taking into account the substrate-induced conformational changes, it should also be ordered. These trypanosome enzymes illustrate how a glycosidase scaffold can achieve efficient glycosyltransferase activity critical for the parasite survival and provide a framework for structure-based drug design.

Keywords: **CRUZI SIALIC ACID DRUG DESIGN**