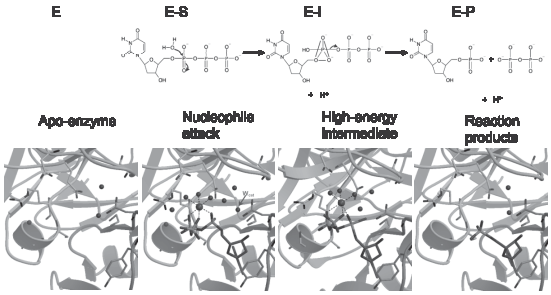
s1.m7.o4 **Experimental Observation of the Pentacovalent Intermediate in dUTPase: Structural Snapshots Along the Reaction Coordinate.** Orsolya Barabás,[ab] Veronika Pongrácz,[a] Júlia Kovári,[a] Matthias Wilmanns[c] and Beáta G. Vértessy[a], [a]*Institute of Enzymology, BRC, Hung. Acad. Sci., Budapest, Karolina út 29-31, H-1113, Hungary,* [b]*Department of Theoretical Chemistry, Eötvös Loránd University, Budapest, H-1117, Hungary, and* [c]*EMBL, Hamburg Outstation, Hamburg, D-22603, Germany. E-mail: vertessy@enzim.hu*

Keywords: Pentacovalent Phosphorous Intermediate; Enzyme Mechanism; dUTPase

The central dogma of enzymology states that the catalytic power of enzymes is due to stabilization of high-energy transition states. Here we provide direct structural evidence for this long-held notion. A series of structural snapshots along an enzymatic phosphate ester hydrolysis identifies a mechanism of significant associative character. We determined the high-resolution crystal structures of substrate-, high-energy intermediate-, and product-complexes of dUTPase. Stepwise comparisons among the presently determined structures and the structure of the apo-dUTPase reveal in clear details how an enzyme responsible for maintaining DNA integrity functions. Substrate hydrolysis is initiated via in-line nucleophile attack of a water molecule oriented by an activating aspartate residue that leads to formation of an additional covalent bond on the α-phosphorous. Stabilization of the hyperbonded penta-covalent intermediate is achieved by i) modulation in the interaction pattern with catalysis-assisting Mg^{2+}, ii) a concerted motion of residues from three conserved enzyme motifs, and, consequently, iii) a remodelling of water hydrogen-bonding network.

Despite a breadth of single-snapshot structural data available on macromolecules, very little is known about the structural changes along the reaction coordinate as an enzyme-catalyzed reaction proceeds. Structures of enzyme-bound products, or non-reactive mimics of transition states and substrates are common. High-energy transition states and intermediates (with incomplete bonding or with hyperbonding), however, usually cannot be rendered for a detailed structural analysis due to their zero lifetimes. Still, in the present study favourable conditions resulting in substantially increased half-life of a hyperbonded intermediate allowed determination of its high-resolution three-dimensional crystal structure in complex with dUTPase. This study therefore presents a true structure-based mechanistic description for enzyme-catalyzed phosphate ester hydrolysis. The enzyme-intermediate complex structure also provides useful insights for structure-based drug discovery, since the optimal lead molecule is the presented transition-state mimicking structure.



s1.m7.o5 **Crystal Structure of Catalase-Peroxidase from *Mycobacterium tuberculosis*.** Katherine A. Brown, Thomas Bertrand, Nigel A.J. Eady and Jamie N. Jones, *Department of Biological Sciences, Centre for Molecular Microbiology and Infection, Flowers Building, Imperial College London, London SW7 2AZ UK. E-mail: k.brown@imperial.ac.uk*

Keywords: Catalase-Peroxidase, Tuberculosis, Isoniazid, Drug Resistance

The *Mycobacterium tuberculosis* CP (*mt*CP) has been the subject of numerous studies as this heme-dependent enzyme is known to activate isoniazid (INH), a core compound used to treat tuberculosis. In particular, it has long been observed that INH resistance in tuberculosis-causing mycobacteria has often been correlated with reduced levels of catalase activity. Subsequently, it was confirmed that the presence of an active CP, encoded by a single gene, *kat*G, is sufficient to confer INH sensitivity in *M. tuberculosis*, the organism principally responsible for tuberculosis. Studies using *mt*CP obtained either from the organism or in a recombinant form demonstrated that the enzyme is capable of oxidizing INH; however, the mechanism of oxidation and the precise mode of action of the drug are still subjects for debate. We have crystallized the enzyme and now report its crystal structure refined to 2.4-Å resolution. The structure reveals new information about dimer assembly and provides information about the location of residues which may play a role in catalysis including candidates for protein-based radical formation. Comparative computational and NMR studies have been used to predict a binding site for INH. ana a proposed enzyme-catalyzed reaction mechanism for activation of the drug.