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.