ESRF	**Experiment title:** Structural studies on species-specific differences among dUTPases	**Experiment number**: MX 18
Beamline: ID 14-4	**Date of experiment**: from: 18 Mar 2003 to: 19 Mar 2003	**Date of report**:
Shifts: 3	**Local contact(s)**: Edward Mitchell	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Orsolya Barabas[1,2*]

Julia Kovari[2]

Veronika Harmat[1*]

[1] Lorand Eotvos University, Institute of Chemistry, Budapest, Hungary

[2] Institute of Enzymology, Hungarian Academy of Sciences, Budapest, Hungary

Report:

Two papers were published based on experiments of the proposal:

PDB code: 1RNJ

Crystal structure of inactive mutant dUTPase complexed with substrate analogue imido-dUTP

Orsolya Barabás, Veronika Pongrácz, Júlia Kovári, Matthias Wilmanns, and Beáta G. Vértessy
"Structural Insights into the Catalytic Mechanism of Phosphate Ester Hydrolysis by dUTPase"
J. Biol. Chem. **279**: 42907-42915 (2004) doi:10.1074/jbc.M406135200

Abstract dUTPase is essential to keep uracil out of DNA. Crystal structures of substrate (dUTP and α,β-imino-dUTP) and product complexes of wild type and mutant dUTPases were determined to reveal how an enzyme responsible for DNA integrity functions. A kinetic analysis of wild type and mutant dUTPases was performed to obtain relevant mechanistic information in solution. Substrate hydrolysis is shown to be initiated via in-line nucleophile attack of a water molecule oriented by an activating conserved aspartate residue. Substrate binding in a catalytically competent conformation is achieved by (i) multiple interactions of the triphosphate moiety with catalysis-assisting Mg^{2+}, (ii) a concerted motion of residues from three conserved enzyme motifs as compared with the apoenzyme, and (iii) an intricate hydrogen-bonding network that includes several water molecules in the active site. Results provide an understanding for the catalytic role of conserved residues in dUTPases.

PDB code: 1ZJK

Crystal structure of the zymogen catalytic region of human MASP-2

Péter Gál, Veronika Harmat, Andrea Kocsis, Tünde Bián, László Barna, Géza Ambrus, Barbara Végh, Júlia Balczer, Robert B. Sim, Gábor Náray-Szabó, and Péter Závodszky
”A True Autoactivating Enzyme: Structural Insight into Mannose-Binding Lectin-Associated Serine Protease-2 Activations”
J. Biol. Chem. **280**: 33435-33444. (2005) doi:10.1074/jbc.M506051200

Abstract Few reports have described in detail a true autoactivation process, where no extrinsic cleavage factors are required to initiate the autoactivation of a zymogen. Herein, we provide structural and mechanistic insight into the autoactivation of a multidomain serine protease: mannose-binding lectin-associated serine protease-2 (MASP-2), the first enzymatic component in the lectin pathway of complement activation. We characterized the proenzyme form of a MASP-2 catalytic fragment encompassing its C-terminal three domains and solved its crystal structure at 2.4 Å resolution. Surprisingly, zymogen MASP-2 is capable of cleaving its natural substrate C4, with an efficiency about 10% that of active MASP-2. Comparison of the zymogen and active structures of MASP-2 reveals that, in addition to the activation domain, other loops of the serine protease domain undergo significant conformational changes. This additional flexibility could play a key role in the transition of zymogen MASP-2 into a proteolytically active form. Based on the three-dimensional structures of proenzyme and active MASP-2 catalytic fragments, we present model for the active zymogen MASP-2 complex and propose a mechanism for the autoactivation process.