Structural and mechanistic study of dUTPases reveals the catalytic roles ofan arginine finger-like residue

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The dUTPase enzymes catalyse the hydrolysis of dUTP to dUMP. In this way dUTPase removes dUTP from the deoxynucleotide pool, avoiding U to be misincorporated in DNA, and produces dUMP that is a precursor of dTTP. Inhibition of dUTPase produces an increment of the dUTP/dTTP ratio in the nucleotide pool resulting in increased uracil content of DNA that activates a hyperactive futile cycle of DNA repair.

dUTPase is a trimer of identical subunits containing one active site each. The C-terminal arm of each monomer determines the catalytic efficiency and contribute to selectivity of the enzyme. This segment contains a conserved Arg directly preceding a glycine-rich P-loop-like motif. Numerous studies demonstrated that the presence of this conserved arginine together with the P-loop-like motif are critical for optimal catalytic efficiency.

In the present study, we focus on the role of the conserved Arg in the mechanism of the trimeric dUTPase from Mycobacterium tuberculosis. As this Arg governs interprotomer catalysis while being located on a distant loop, we propose that it conceivably meets the requirements established for Arg fingers. We address its contribution to the catalytic mechanism of Mycobacterium tuberculosis dUTPase by investigating constructs of with either deletion of the P-loop-like motif or exchange of the conserved arginine. The structural data from crystallography and molecular dynamics simulations together with kinetic and ligand binding analyses reveal the unique role for the Arg in active site organization and providing optimal ligand geometry for catalysis. QM/MM calculations are performed to quantitatively assess the structural and electrostatic contributions of the Arg in catalysis.