Surveillance of the nucleotide pool: insights into the catalytic mechanism of mycobacterial antimutator protein MutT2

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Mis-incorporation of modified nucleotides, such as 8-oxo-dGTP or 5-methyl-dCTP, in DNA can be detrimental to genomic integrity. MutT proteins are sanitization enzymes which function by hydrolyzing such nucleotides and regulating the pool of free nucleotides in the cytoplasm. Mycobacterial genomes are found to have a set of four MutT homologs, namely, MutT1, MutT2, MutT3 and MutT4. Mycobacterial MutT2 can hydrolyze dCTP, dTTP, 5m-dCTP and 8-oxo-dGTP directly to their respective mono-phosphate products. MutT2 has also been associated with high drug resistance in *M. tuberculosis* W. Beijing strains. Here, we report biochemical studies and high-resolution crystal structures of *M. smegmatis* MutT2 and its complexes, determined at resolutions ranging from 1.10Å to 1.73Å. Intriguingly, the native protein and its complexes involving products crystallize in space group $P2_12_12$, while those involving substrates crystallize in space group $P2_1$. Each molecule has an α/β/α sandwich fold arrangement, comprised of 8 β-strands organized into two mixed β-sheets, two α-helices and two 3_{10} helices. The nucleoside moiety of the ligands is similarly located in all the complexes, while the location of the remaining tail exhibits variability. The high specificity towards cytosine bases is provided by a critical Asp (D116) residue. Conservation of water and metal ion positions in the active site provides insights into the recognition and mechanism of action of this enzyme. The work presented here is the first report of a MutT2-type protein in complex with ligands.