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THE CRYSTAL STRUCTURES OF *ESCHERICHIA COLI* MutT PROTEINS WITH AND WITHOUT MANGANESE IONS

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8-Oxoguanine is produced in the chromosomal DNA, RNA and free nucleotides such as dGTP and GTP by active oxygen species usually formed during cellular metabolic processes. 8-Oxoguanine can mispair with adenine during replication and transcription. *Escherichia coli* MutT protein prevents replicational and transcriptional errors by hydrolysis of potent mutagenic substrates for DNA and RNA syntheses, 8-oxo-dGTP and 8-oxo-GTP to the corresponding nucleoside monophosphates. Our research purpose is to get detailed insights into the specific recognition for the 8-oxoguanine base and the catalytic mechanism for the hydrolysis of 8-oxo-dGTP and 8-oxo-GTP by the MutT protein. In this study, we have determined the crystal structures of E. coli MutT proteins with and without manganese ions. First, the structure of apo MutT was solved by the MAD method using selenomethionyl MutT protein. Second, the structure of MutT-metal complex was solved by the molecular replacement method using the apo MutT structure. The overall structure of MutT consists of an α + β fold with 6-stranded β-sheet sandwiched between the two α-helices. Three manganese ions bind to a glutamate cluster in Nudix motif ($GX_5EX_7REUXEEXGU$) with no significant conformational changes compared with apo MutT.

Keywords: 8-OXO-DGTPase MUTATOR NUDIX FAMILY

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STRUCTURE ANALYSIS OF IPMI SMALL SUBUNIT-EFFECT OF INTERMOLECULAR DISULFIDE BOND ON CRYSTAL QUALITY

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Leucine biosynthesis is carried out with three steps. In the first step, 2-oxoisovaleric acid and acetyl-CoA are condensed into 2-isopropylmalic acid by isopropylmalate synthase (IPMS). The second step is the interconversion between 2-isopropylmalate and 3-isopropylmalate. At the third step, 3-isopropylmalate is changed to 2-oxoisocaproic acid by isopropylmalate dehydrogenase (IPMDH). Isopropylmalate isomerase (IPMI) catalyzes the pathway-specific reaction at the second step, and it is composed of two different subunits that are coded for by two genes of the leucine operon. We have determined the crystal structure of small subunit of IPMI from *Pyrococcus horikoshii* (phoIPMI-S) by Se-Met MAD method. Two crystal forms, orthorhombic ($P2_12_12_1$) and hexagonal ($P6_222$) of phoIPMI-S were obtained with and without reducing agent (DTT), respectively. While the crystal of orthorhombic form reflected to 2.5 Å resolution, hexagonal form reflected to 1.98 Å. The crystal structures obtained from two crystal forms show that only hexagonal form has intermolecular disulfide bonds between pairs of C-terminal cysteine residues which related by 2-fold axis of crystal symmetry. Thus those intermolecular disulfide bonds seem to contribute to stabilization of crystal packing and enhance crystal quality. The final refined structure of phoIPMI-S reveals that it consists of α- and β- domains and is most similar to C-terminal domain of aconitase which catalyzes dehydration of citrate to form isocitrate in the Krebs cycle. Structural comparison between phoIPMI-S and aconitase suggests that the specific arrangement of large and small subunits of IPMI is similar to that of N- and C- domains in aconitase.

Keywords: MAD, ISOMERASE, CRYSTAL QUALITY

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HUMAN MITOCHONDRIAL BRANCHED CHAIN AMINOTRANSFERASE : AN X-RAY CRYSTALLOGRAPHIC STUDY

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Human mitochondrial branched chain aminotransferase (hBCATm) is a pyridoxal phosphate (PLP) dependent enzyme which catalyzes reversible transamination of branched chain L-amino acids to their respective α-keto acids. The crystal structures of hBCATm, in the PLP and ketimine (with isoleucine) forms, and the PLP form of a mutant in which the two reactive cysteines have been mutated to alanine (C315A/C318A) have been determined. Under oxidizing conditions, these cysteines form a disulfide bond inhibiting the enzyme. In the native crystals the enzyme is active. The cysteines are located in a β-turn (315-318), preceded by yet another β-turn (311-314). Their side-chain distances and angles suggest that the sulfurs participate in a thiol-thiolate hydrogen bond. In the mutant structure this bond cannot form, and there are changes in the backbone as well as side-chain conformations and altered hydrogen bonding at the β-turns. A hydrogen bond seen between the amide nitrogen at Ala315 and carbonyl oxygen at Gly312 is broken and a new one is formed between the side chain of Gln316 and the carbonyl oxygen at Thr313. In the PLP and the ketimine structures, Thr313 is a crucial residue involved in cofactor phosphate and substrate carboxylate stabilization. Further the inter-domain loop (171-181), which controls the access to the active site has shifted in the mutant structure such that the active site is open. It is well ordered and less mobile in both the monomers in this structure whereas it is disordered in one of the monomers of the native structures. (Funding: NIH Grant DK34738)

Keywords: **ENZYME, BRANCHED CHAIN AMINOTRANSFERASE, TRANSAMINASE**

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HIGH RESOLUTION STRUCTURES OF WILD TYPE AND APO HUMAN CuZn SUPEROXIDE DISMUTASE AND ITS FALS-RELATED MUTANTS

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CuZn superoxide dismutases (SODs) (EC 1.15.1.1) form a class of metalloenzymes which catalyse the dismutation of the superoxide radical anion into oxygen and hydrogen peroxide. SOD forms a crucial part of the cellular defense against oxidative stress. Here, we present the high-resolution crystal structures of human superoxide dismutase in the wild-type, reduced and apo forms. We also present the crystal structures of a number of SOD mutations implicated in motor neuron disease (amyotrophic lateral sclerosis). The relationship between structure and function in SOD will be discussed in terms of this fulminant disease.

Keywords: **SUPEROXIDE DISMUTASE FALS Cu Zn PROTEIN**