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Crystal Structure of hMTH1 in Complex with its Reaction Product, 8-oxo-dGMP

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8-Oxoguanine (8-oxoG) generated in the chromosomal DNA, RNA and free nucleotides by reactive oxygen species has high mutagenic potency due to its mispairing with adenine. *E.coli* MutT, which hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP, prevents the misincorporation of 8-oxoG into DNA and the subsequent A:T to C:G transversion mutations. The mammalian counterpart of MutT, MutT homolog-1 (MTH1) can hydrolyze 8-oxo-dGTP less efficiently than MutT and a variety of oxidized purine nucleoside triphosphates. It is interesting to elucidate the structural basis for the difference in substrate-specificity between MutT and MTH1.

In this work, we have determined the crystal structure of hMTH1 (human MTH1) complexed with a product, 8-oxo-dGMP. The binding mode of 8-oxo-dGMP in the substrate-binding pocket of hMTH1 is quite different from one found in MutT-8-oxo-dGMP complex. It reveals the difference in preference of 8-oxo-dGTP to a normal nucleotide dGTP between hMTH1 and MutT. The structure of hMTH1-8-oxo-dGMP complex provides implications for the recognition mechanism of the most effective substrate, 2-OH-dATP by hMTH1.

Keywords: DNA repair, substrate-specificity, hydrolysis

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The Crystal Structure of L-proline Dehydrogenase in a Hyperthermophilic Archaeon

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Two different type of archaeal L-proline dehydrogenase (PDH) was found in *P. horikoshii* OT-3. PDH1 formed an operon that consisted of two consecutive genes, PH1363 and PH1364 and the molecular masses of αand β subunit were determined to be about 56 and 43kDa, respectively. The native molecular mass of PDH1 is 440kDa with (αβ4 hetero-octamer.

We have purified recombinant PDH1, crystallized and determined the crystal structure of PDH1 from *P. horikoshii* at 2.8Å resolution. The structure revealed that β subunit, which bears PDH activity, was similar to monomeric sarcosine oxidase. FAD was bound in the β subunit non-covalently. The α subunit contains a dinucleotide fold domain with unexpected ATP, a central domain, a N-terminal domain and Cys-clustered domain. Furthermore FMN is bound between α and β subunits. The structure of αand β subunit is totally different except the dinucleotide domain, but it is suggested that each structure diverged from common ancestral flavoenzyme formed a complex to make a new electron transport pathway.

Keywords: dehydrogenase, flavoenzymes, complex structure

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Dual Substrate Recognition of Acetylornithine Aminotransferase

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Acetylornithine aminotransferase (AcOAT) is a pyridoxal 5'-phosphate(PLP)-dependent enzyme. The enzyme catalyses the fourth reaction on the arginine biosynthetic pathway, AcoAT reversibly catalyze the transamination reaction in which the α-amino group of N-acetyl-L-ornithine is transferred to N-acetyl-L-glutamate ν-semialdehyde to produce 2-oxoglutarate and L-glutamate. AcOAT is distinguished from other typical aminotransferases in that the δ-amino group of acetylornithine forms a Schiff base with the cofactor PLP, although glutamate forms a Schiff base between its α-amino group and the cofactor. This implies that the α-carboxylate and N-acetyl group of acetylornithine and the γ-carboxylate of glutamine are on the phosphate side of the cofactor.

The crystal structure of native AcOAT from *Thermus thermophilus* HB8 and its complexes N-(5'- phosphopyrydoxyl) -N-acetylornithine and N-(5'-phosphopyrydoxyl)- L-glutamate have been solved and refined to *R*-factors 19.5, 22.6, and 18.1% at 1.35, 2.05, and 2.25Å. No significant change in the overall structure in AcOAT was observed on binding of the ligands. The active site residues do not show any significant changes in side-chain conformations except for Phe140 and Glu197.

Keywords: X-ray crystallography of biological macromolecules, substrate binding, aminotransferases

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Detection of the Conformational Changes of FAD During the Catalysis

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BphA4 is the ferredoxin reductase component of biphenyl dioxygenase from *Pseudomonas* sp. strain KKS102. In order to provide a structural basis for discussing the electron transport mechanism between ferredoxin and ferredoxin reductase, we determined the crystal structures of BphA4 and its NADH complex (blue semiquinone form) at 1.5Å and 1.6Å resolution respectively.

The crystal of the BphA4-NADH complex were prepared by the soaking method. The crystal color gradually changed from yellow to blue within 2 hours, and then the crystal was frash-frozen. Data collections were carried out at NW12 of PF (Tsukuba). The electron density of the blue semiquinone form shows that the nicotinamide ring of NADH is located beside the isoalloxazine ring of FAD. The difference Fourire map shows a bent of the isoalloxazine ring and a flip of the ribitol part of FAD in the blue-semiquinone form. These conformational changes seem to be caused by the conformational change of the N10 atom of FAD, which seems to have a non-planar conformation in the blue-semiquinone form.

Keywords: flavoprotein, reactive intermediate, conformational change

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Crystal Structure of Biphenyl Dioxygenase

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Biphenyl dioxygenase is the enzyme that catalyzes the stereospecific dioxygenation of the aromatic ring. This enzyme has attracted the attention of researchers due to its ability to oxidize polychlorinated biphenyls (PCBs), which is one of the serious