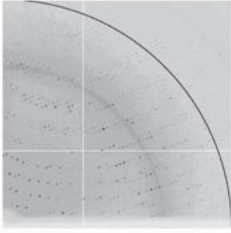


Diffraction image of <i>B. pallidus</i> D-AI		Data collection statistics	
	Resolution range (Å)		2.3
	No. of measured reflections	592,546	
	No. of unique reflections	91,188	
	Completeness (%)	100	
	Mean $I/\sigma(I)$	8.8	
	R_{merge} (%)	0.075	
	Space group	$P2_12_12$	
	Cell dimensions	a (Å)	144.9
		b (Å)	127.9
	c (Å)	109.5	

Keywords: isomerase, rare sugars, X-ray structure

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Structures of NADH and NAD⁺ bound 3 α -hydroxysteroid dehydrogenase from *Pseudomonas* sp. B-0831

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Bacterial 3 α -hydroxysteroid dehydrogenase (3 α HSD) catalyzes the reaction of the reversible inter-conversion between 3 α -hydroxysteroid and 3-ketosteroid in a coenzyme dependent manner. We reported the structure of 3 α HSD of *Pseudomonas* sp. B-0831 (Ps3 α HSD) complexed with NADH (*JBC*, 2006, **281**, 31876-84). The crystal which was obtained by co-crystallization with NADH, contained a dimer in an asymmetric unit of which one is apo-form and the other is holo-form. There was a distinct difference in the so-called substrate-binding loop (185-207) between these two subunits i.e. it consists of two α -helices in the holo-form while it is in disordered form in the apo-form. Here we obtained the complex by co-crystallization with NAD⁺, which contained a dimer in an asymmetric unit as well. In this case, the coenzyme was bound to the both monomers in the dimer. It is noteworthy that there was a conformational difference between these subunits. One of them has two α -helices in the so-called substrate-binding loop region, while the other, even though NAD⁺ was bound, takes a disordered form similar to the NADH complex. In these two alpha helices, there was a difference found in their mutual spatial arrangements between the respective complexes with NADH and NAD⁺. This difference which accompanies a shift of the hydrogen bond partner of Tyr200 from His150 in the complex with NADH to Val74, whereas with NAD⁺ results in loss of hydrogen bonding found between Tyr153 and NADH. This conformational change might play an important role in the coenzyme recognition of Ps3 α HSD depending on the redox state.

Keywords: short-chain dehydrogenase reductases, cofactors, dehydrogenase steroid nucleotide

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High-resolution X-ray diffraction study of the hMTH1 mutant

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Human MutT homolog-1 (hMTH1) hydrolyzes a variety of oxidized nucleoside triphosphates to their corresponding monophosphates and prevents replicational and transcriptional errors caused by their misincorporations into DNA and RNA. hMTH1 has broad substrate specificity for several oxidized purine nucleotides such as 8-oxo-dGTP, 2-oxo-dATP and 8-oxo-dATP. Recently, we have determined the crystal structures of hMTH1 complexed with 8-oxo-dGTP and 2-oxo-dATP, respectively. These complex structures have revealed that hMTH1 recognizes the different oxidized purine nucleotides, 8-oxo-dGTP and 2-oxo-dATP, by the exchange of the protonation site in the neighboring Asp residues. To our knowledge, this is a brand new mechanism for the broad substrate specificity of enzymes. In order to completely establish this mechanism by the protein crystallography, it is essential to identify the protonation states of these two Asp residues by ultrahigh-resolution crystal structures of hMTH1. We have succeeded to obtain crystals which diffract to better than 1.12 Å resolution using the hMTH1 mutant with a homogeneous N-terminus and collect the diffraction data at 1.23 Å resolution. In this structure, two molecules in the asymmetric unit interact with each other through their mutated N-terminal regions, and a crystallization reagent, imidazole, is bound to Trp, Asp and Asn residues which are important for the substrate recognition. The high-resolution structure of hMTH1-imidazole complex has revealed the detailed recognition scheme of imidazole by the stacking interaction with Trp and hydrogen bonding interactions with Asp and Asn. Now the refinements with anisotropic thermal parameters using *SHELX* are in progress.

Keywords: DNA repair enzymes, high-resolution protein structures, substrate binding

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Tertiary structure of the catalytic and chitin-binding domains of hyperthermophilic chitinase

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A chitinase, engineered from the genes PF1233 and PF1234 of *Pyrococcus furiosus*, is a hyperthermophilic glycosidase that effectively hydrolyzes both α and β crystalline chitin. This chitinase contains two catalytic domains (AD1 and AD2) and two chitin-binding domains (ChBD1 and ChBD2). We determined the crystal structures of ChBD2 and AD2 (to the resolution at 1.7 Å and 1.5 Å, respectively), which are important for the activity of the chitinase toward crystalline chitin. The structure of ChBD2 comprised a typical β -sandwich architecture, which consists of two four-stranded β -sheets, and was similar to that of other carbohydrate-binding module 2 family proteins, despite low sequence similarity. The chitin-binding