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

Teruya Nakamura[a], Yuki Kitaguchi[a], Masayuki Miyazawa[a], Sachiko Toma[a], Shinji Ikemizu[a], Masahiro Shirakawa[b], Yusaku Nakabeppu[c], Yuriko Yamagata[a], *[a]Graduate School of Pharmaceutical Sciences, Kumamoto University, Japan. [b]Graduate School of Engineering, Kyoto University, Japan. [c]Medical Institute of Bioregulation, Kyushu University, Japan.* E-mail: 040y9002@pharm.stud.kumamoto-u.ac.jp

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

Hideaki Tsuge[1], Ryushi Kawakami[2], Haruhiko Sakuraba[2], Kenji Aki[1], Nobuhiko Katunuma[1], Hideo Ago[3], Masashi Miyano[3], Toshihisa Ohshima[2], *[1]Institute for Health Sciences,Tokushima Bunri University, Japan. [2]Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, Japan. [3]Riken HARIMA, Japan*. E-mail: tsuge@tokushima.bunri-u.ac.jp

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

Ikuko Miyahara[a,b] , Mitsuyoshi Matsumura[a,b], Masaru Goto[a,b], Rie Omi[a,b], Ken Hirotsu[a,b], Hiroyuki Mizuguchi[c], Hideyuki Hayashi[c], Hiroyuki Kagamiyama[c], *[a]Graduate School of Science, Osaka City University. [b]Harima Institute/SPring-8, The Institute of Physical and Chemical Resaech(RIKEN) Hyogo, Japan. [c]Osaka Medical School, Japan.* E-mail: miyahara@sci. osaka-cu.ac.jp

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

Miki Senda[a], Shinya Kishigami[b], Shigenobu Kimura[b], Masao Fukuda[c], Toshiya Senda[d], *[a]Japan Biological Information Research Center (JBIRC), Japan Biological Informatics Consortium (JBIC), Tokyo, Japan. [b]Graduate School of Life Science, University of Hyogo, Hyogo, Japan. [c]Nagaoka University of Technology, Niigata, Japan. [d]Biological Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan.* E-mail: msenda@jbirc.aist.go.jp

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

Toshiya Senda[a], Yutaka Furusawa[b,c], Venugopalan Nagarajan[d], Masaru Tanokura[c], Masao Fukuda[d], *[a]Biological Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan. [b]Japan Biological Information Research Center (JBIRC), Japan Biological Informatics Consortium (JBIC), Tokyo, Japan. [c]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan. [d]Nagaoka University of Technology, Niigata, Japan.* E-mail: tsenda@jbirc.aist.go.jp

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

environmental contaminants. Here we present the crystal structure of the terminal oxygenase component of the biphenyl dioxygenase (BphA1A2) derived from *Rhodococcus* strain sp. RHA1. This is the first crystal structure of the biphenyl dioxygenase. We also determined the crystal structure of the BphA1A2-biphenyl complex. Structural comparison between the substrate free and complex forms revealed that the substrate-binding pocket makes significant conformational changes upon substrate binding to accommodate the substrate into the pocket. The analysis with the two crystal structures suggested that the residues in the substrate-binding pocket can be classified into three groups, which respectively seem to be responsible for the catalytic reaction, the orientation/conformation of the substrate, and the conformational changes of the substrate-binding pocket.

Keywords: oxygenase, substrate binding, conformational change

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Crystal Structures of Serine Racemase from *S. pombe*

Masaru Goto[a,b], Rie Omi[a], Ikuko Miyahara[a], Ken Hirotsu[a], [a]*Graduate school of Science, Osaka City University, Osaka, Japan.* [b]*Department of Biochemistry, Osaka Medical College, Takatsuki, Japan.* E-mail: goto@sci.osaka-cu.ac.jp

Free D-serine has been found to be confined predominantly to the forebrain structure of mammals and persists at high levels throughout embryonic and postnatal life. Several reports strongly suggest that serine racemase exits in mammalian brain and produces D-serine, an endogenous ligand for *N*-Methyl-D-Aspartate receptor. Serine racemase requires pyridoxal 5'-phosphate and catalyzes racemization from L-serine to D-serine. Surprisingly, the distribution of putative serine racemase is not limited to higher animals. Mouse serine racemase might fall on a new class of pyridoxal amino acid racemases, which is distinct from both bacterial and fungal alanine racemases, but is similar to bacterial threonine dehydratase in primary structure.

S. pombe gene homologous with mouse serine racemase is overexpressed in *E. coli*. The enzyme is purified and crystallized. Three-demensional structures of serine racemase in its unliganded form and complexed with the AMP-PCP of activator have been determined at 1.7 and 1.9 Å resolution, respectively. The enzyme is a homodimer, and each subunit is divided into small and large domains. Based on its folding, the enzyme belongs to fold type II. Interestingly, these structures show that an activator is bound at the subunit interface away from the active site PLP.

Keywords: three-dimensional protein structure, enzyme activity mechanism, racemases

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Crystal Structures of Formaldehyde Dehydrogenase Complexed with Inhibitors

Tomonobu Umeda[a], Yoshio Kusakabe[a], Nobutada Tanaka[a], Kiyoshi Ito[b], Tadashi Yoshimoto[b], Kazuo T. Nakamura[a], [a]*School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan.* [b]*School of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.* E-mail: t-ume@pharm.showa-u.ac.jp

Formaldehyde dehydrogenase from *Pseudomonas putida* (PFDH) is a member of the zinc-containing medium-chain alcohol dehydrogenase family. The pyridine nucleotide NAD(H) in PFDH, which is distinct from the coenzyme (as cosubstrate) in typical alcohol dehydrogenases (ADHs), is tightly but not covalently bound to the protein and acts as a cofactor. PFDH can catalyze aldehyde dismutations without an external addition of NAD(H). The crystal structure of PFDH was solved by the multiwavelength anomalous diffraction method using intrinsic zinc ions and was refined at a 1.65Å resolution [1].The 170-kDa homotetrameric PFDH molecule showed 222-point group symmetry. Although the secondary structure arrangement and binding mode of catalytic and structural zinc ions in PFDH were similar to those of typical ADH, a number of loop structures that differ between PFDH and ADHs in their lengths and conformations were observed.

Here we have determined the crystal structures of PFDH complexed with pyrazole inhibitors.

[1] Tanaka N., Kusakabe Y., Ito K., Yoshimoto T., Nakamura K.T., *J. Mol. Biol.*, 2002, **324**, 519.

Keywords: crystal structure, formaldehyde dehydrogenase, alchol dehydrogenase

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Molecular Basis for Tay-Sachs Revealed by the Crystal Structure of Human β-HexosaminidaseA

M. Joanne Lemieux[1], Brian L. Mark[1,2], Maia M. Cherney[1], Spencer Knapp[3], Don L. Mahuran[4], Michael N.G. James[1], [1]*Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.* [2]*Present address: Department of Microbiology, University of Manitoba, Winnipeg, MB, Canada.* [3]*Department of Chemistry, Rutgers, New Brunswick NJ, USA.* [4]*Hospital for Sick Children, University of Toronto, Toronto, ON, Canada.* E-mail: mlemieux@ualberta.ca

β-hexosaminidase-A (HexA) is essential for the degradation of G_{M2} glycolipids in the central and peripheral nervous system. Accumulation of G_{M2} leads to neurodegeneration associated with Tay-Sachs and Sandoff disease. Here we present the X-ray crystallographic structure of human lysosomal HexA to 3.0Å resolution. The structure reveals a heterodimer consisting of an α- and a β-subunit. The dimer interface creates two distinct active sites: and α(β) and β(α); only the α(β) site can hydrolyse G_{M2} whereas both sites can hydrolyse oligomers of NAG. We also present a second structure of HexA with a NAG-thiazoline, a transition state substrate analog, bound in the active site. Hex A, a member of Family 20 of the glycosyl hydrolases, makes use of substrate-assisted catalysis in the removal of the non-reducing sugar from an oligosaccharide. The intermediate in the reaction pathway of G_{M2} is a cyclic oxazolinium formed by the acetyl group on C2' of the non-reducing β(1,4)-GalNAc of the substrate. In both active sites, the general acid is a Glu residue and the positive charge of the oxazolinium ring is stabilized by the negative charge on an Asp residue. In the α(β) site, G_{M2} binding is promoted by Arg424 and Asn425 residues. The β(α)-site lacks these key residues but has Leu453 and Asp452 in their place that would repel the negatively charged sialic acid of G_{M2}.

Keywords: enzyme mechanisms, glycosyl hydrolases, neurobiology

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Structural Basis for the Induced Fit, Substrate Recognition, and Mechanism of Threonine Synthase

Rie Omi[1,2], Masaru Goto[1], Ikuko Miyahara[1,3], Ken Hirotsu[1,3], [1]*Graduate School of Science, Osaka City University, Japan.* [2]*Institute for Chemical Research, Kyoto University, Japan.* [3]*The Harima Institute/SPring8, the Institute of Physical and Chemical Research, Japan*. E-mail: omi@sci.osaka-cu.ac.jp

Threonine synthase, which is a pyridoxal 5'-phosphate dependent enzyme, catalyzes the β, γ-replacement reaction of an *O*-phospho-L-homoserine to give a threonine and an inorganic phosphate. The three-dimensional structures of the enzyme from *Thermus thermophilus* HB8 in its native form and complexed with the substrate analogue (2-amino-5-phosphonopetanoic acid) have been determined at 2.15 and 2.0 Å resolution, respectively. The enzyme is a homo dimer, with the polypeptide chain of the subunit folded into large, small, and swap domains. The complexed form of the enzyme assigned as an enamine uncovered the interactions of the cofactor-analogue conjugate with the active-site residues. The binding of the substrate analogue induces the large conformational change at the domain level to close the active-site. The small domain rotates by about 25° and approaches the large domain to shield the substrate analogue from the solvent region. The complicated catalytic process of the enzyme has been elucidated based on the complex structure to reveal the stereochemistry of the reaction and present the released inorganic phosphate as the possible catalyst to