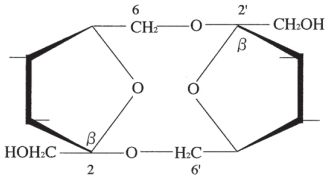
fructose, with a terminal glucose residue, and is found in some plants and bacteria. This polysaccharide serves as storage carbohydrates, and has shown to play a role in a tolerance for drought and low temperature in plants. In some microorganisms, levan is enzymatically degraded into several forms of oligosaccharides. Bacterial levan fructotransferase (LFTase) has been known to produce a cyclic difructose, β-D-fructose-2',6:2,6'-dianhydride (DFA IV; see chemical structure below) from levan. It is likely that LFTase catalyzes an intramolecular fructosyl transfer reaction of levan to produce DFA IV, although its mechanistic details are still elusive. DFA IV has been suggested to be a useful sugar alternative for diabetic patients, mainly due to its physiological properties which are indigestible but not absorbed in the intestine of mammals. With these features, production of DFA IV has gained attentions from food industry. DFA IV can be chemically synthesized via multistep reactions, but this traditional method exhibits a lower efficiency of yield, in particular in forming the 2,6':2',6 linkage of cyclic difructose. Therefore, its production by a biological reaction with an enzyme has an advantage over the traditional chemical synthesis. To this end, structural and mechanistic details for LFTase are required to be investigated.

Recently, we crystallized a bacterial LFTase, and its structure is now determining by MAD data using a selenomethionine-labeled protein crystal. Crystal of LFTase was diffracted to 2.6 Å resolution and belongs to a space group of $P2_12_12_1$, with cell dimensions of a=81.9 Å, b=166.6 Å, c=261.9 Å and α=90°, β=90°, and γ=90°. There are tetramer in an asymmetric unit. We will describe structural feature of LFTase and a possible mechanism for catalysis

Chemical structure of DFA IV



Keywords: carbohydrate, transferase, enzyme

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Dissecting enzyme mechanisms in the purine nucleotides biosynthetic pathway

Gota Kawai,[a,b] Seiki Baba,[b,c] Mayumi Kanagawa,[b] Seiki Kuramitsu,[b,d] Gen-ichi Sampei,[b,e] [a]*Faculty of Engineering, Chiba Institute of Technology,* [b]*RIKEN SPring-8 Center, Harima Institute,* [c]*SPring8/JASRI,* [d]*Graduate School of Science, Osaka University,* [e]*Department of Applied Physics and Chemistry, The University of Electro-Communications (Japan).* E-mail: gkawai@sea.it-chiba.ac.jp.

The purine nucleotide biosynthesis proceeds by a 14-step branched pathway. This pathway is common to most organisms, and all reactions are concerned with the formation of C-N bond. And moreover, we can observe some similar reactions in this pathway. Thus, it is important to compare the structure and reaction mechanisms to each other by determining of 3D structure of the enzymes, when the genesis of this pathway is considered.

We determined 32 structures of the enzymes in this pathway from several thermophilic bacteria including *Thermus thermophilus* HB8, *Sulfolobus tokodaii* strain7, *Aquifex aeolicus* VF5, *Geobacillus kaustophilus* HTA426, *Symbiobacterium toebii*, *Methanocaldococcus jannaschii* DSM 2661, and *Thermotga maritima* MSB8; PurD, PurN, PurU, PurS, PurL, PurM, PurK, PurE, PurC, PurB, PurH and GuaA, and 23 structures are in PDB. In addition to the structure determination, we started biochemical analysis as well as molecular dynamics (MD) simulations of several enzymes in this pathway.

In the case of the PurD, glycinamide ribonucleotide synthetase, we determined the structure of *Gk*PurD in complex with glycine which is one of the ligands of this enzyme. The bound glycine located close to the bound ATP so that the γ-phosphate group is ready to attack the carbonyl group as the first stop of the reaction by PurD. By comparing the structures of PurD from *Tt*, *Gk*, *Aa* as well as *Escherichia coli* (1GSO), *Tm* (1VKZ), *Homo sapiens* (2QK4), it was found that the B-domain, which is a part of the ATP-grasp module, can move (open and close) and this movement must be important for the initiation of the reaction.

PurK, N^5-aminoimidazole ribonucleotide synthetase, is quite similar in structure with PurD in spite of the low sequence homology. We determined the crystal structure of TmPurK; four monomers were found in the asymmetric unit and each of monomers has different conformation in the location of the B-domain. This also indicates the movement of the B-domain. Interestingly, the location of ATP is changed according to the location of B-domain; closing the B-domain seems to initiate the reaction.

PurN, glycinamide ribonucleotide transformylase 1, shares the nucleotide binding domain with PurD and PurK. Instead of using ATP and low-molecular carbonyl compound like PurD and PurK, PurN utilizes 10-formyltetrahydrofolate as the carbonyl source. We determined crystal structures of PurN from *Aa*, *Gk* and *Syto*, and the ligands recognition as well as reaction mechanism are now being investigated based on the structural comparison as well as MD simulation.

By comparing structures and reaction mechanisms of these enzymes, we are investigating how this complicated system of reactions was generated.

Keywords: purine, nucleotide, metabolism

MS93.P41

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Crystal structure of aspartate racemase from *Lactobacillus sakei* NBRC-15893

Yasuo Hata,[a,b] Tomomi Fujii,[a] Makoto Ishiyama,[a] Takae Yamauchi,[b] Yoshitaka Gogami,[c] Tadao Oikawa,[c] [a]*Institute for chemical research, Kyoto University (Japan).* [b]*Institute of Sustainability Science, Kyoto University (Japan).* [c]*Department of Life Science and Biotechnology, Faculty of Chemistry, Materials and Bioengineering, Kansai University (Japan).* E-mail: hata@scl.kyoto-u.ac.jp

Aspartate racemase (AspR) catalyzes the interconversion between L- and D-aspartate. AspR belongs to the PLP-independent racemase group and is thought to employ a two-base mechanism to catalyze both the directions of racemization. The enzymes of this group utilize two cysteine residues as the conjugated catalytic acid and base in the catalytic reaction. Only the crystal structure of AspR from a hyperthermophilic archaeon has been reported so far. To elucidate the structure-function relationship of AspR which works in the range of the low to medium temperature, we have determined the crystal structure of AspR from *Lactobacillus sakei* NBRC 15893 (LsAspR).

LsAspR was crystallized at 293 K by the sitting-drop vapour-diffusion method using a precipitant solution containing 25% (v/v) PEG-MME 550, 5% (v/v) 2-propanol and 0.1 M sodium acetate pH 4.8. The approximate dimensions of the obtained crystals were 0.40 × 0.15 × 0.03 mm^3. The crystals belonged to space group $P3_121$ with unit cell parameters of $a = b = 105.5$ Å and $c = 96.5$ Å. They diffracted up to 2.6 Å resolution. The asymmetric unit contained one dimeric molecule of LsAspR with a corresponding crystal volume per protein mass (V_M)

of 2.97 Å^3/Da and a solvent content of 59%. The crystal structure has been determined by molecular replacement. The current model was refined at 2.6 Å resolution to an *R*-factor of 23.8% (R_{free} = 31.6%).

In crystals, LsAspR adopts a homodimeric form. The subunit consists of two domains: the N-terminal domain (residues 1-104 and 216-234) and the C-terminal domain (residues 105-215). In each domain, a central four-stranded parallel β-sheet is flanked by six α-helices. The spatial arrangement of the strictly conserved residues Cys84 and Cys196 strongly indicates that the active site of LsAspR must be located in the cleft between the two domains.

Keywords: racemase, aspartate racemase, crystal structure

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Insights into the fumarase reaction mechanism from crystal structures of Rv1098c

Ariel E. Mechaly,[a] Ahmed Haouz,[b] Isabelle Miras,[b] Pedro M. Alzari,[a] Marco Bellinzoni,[a] [a]*Unité de Biochimie Structurale, Institut Pasteur, Paris (France).* [b]*Plateforme de Cristallogenèse et Diffraction de Rayons X, Institut Pasteur, Paris (France).* E-mail: amechaly@pasteur.fr

Fumarase (fumarate hydratase: EC 4.2.1.2) catalyzes the reversible conversion of L-malate to fumarate and H_2O during the TCA cycle. In Mycobacteria tuberculosis, this reaction is catalyzed by the essential enzyme Rv1098c encoded by the fum gene [1, 2].

Rv1098c belongs to a superfamily of enzymes that catalyze reactions involving fumarate molecules. This superfamily not only includes fumarases but also L-aspartases, δ-crystalline, 3-carboxy-cis,cis-muconate lactonizing enzymes, arginino-succinatases as members. Despite a low sequence identity (~25%), the crystal structures of several members of this superfamily revealed a conserved overall fold.

We determine the crystal structures of Rv1098c in its apo form as well as its complexes with the competitive inhibitor meso-tartrate and the substrate/product malate. These structures reveal that ligand binding promotes conformational changes at the enzyme C-terminal domain that close the active site. Inspection of the active site clefts allowed us to propose a catalytic mechanism through a detailed description of the interactions between substrate and catalytic residues.

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Keywords: mycobacteria, TCA cycle, fumarase

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Cap architecture conservation in monoglyceride lipases from bacteria to humans

C. Sturm,[a] S. Rengachari,[a] G. A. Bezerra,[a] L. Riegler,[a] C. C. Gruber,[b] U. Taschler,[a] A. Boeszoermenyi,[a] R. Zimmermann,[a] K. Gruber,[a] M. Oberer,[a] [a]*Institute of Molecular Biosciences, University of Graz, Graz, (Austria), A-8010* [b]*ACIB GmbH, Petersgasse 14, A- 8010 Graz (Austria).* E-mail: christian.sturm@edu.uni-graz.at

Monoglyceride lipases (MGL) catalyze the hydrolysis of monoglycerides into free fatty acid and glycerol. MGL have been identified in all kingdoms of life and have adopted different substrate specificities with respect to chain lengths, depending on their different roles in the host organisms. In humans, MGL play an integral part in lipid metabolism affecting energy homeostasis and signaling processes. It also hydrolyzes the endocannabinoid 2-arachidonoylglycerol, which regulates a range of physiological processes (e.g. pain sensation). In bacteria, especially short chain monoglycerides are highly toxic. Thus, MGL is crucial for the survival of the bacteria. Despite the biological, pharmacological and biotechnological interest in MGL, the 3D structure of human MGL was the first member of this enzyme family to be solved in 2010. We report the first crystal structures of MGL from a bacterial species (Bacillus sp. H257) in its free form at 1.2Å and covalently complexed to phenylmethanesulfonyl fluoride at 1.8Å resolution, molecular dynamics simulation of bacterial MGL (bMGL, 250 aa) and a comprehensive comparison of the resulting structural insights with the human homolog (303 aa): The core structure is composed of an α/β hydrolase fold. A cap region, which is often observed in α/β hydrolase, is inserted between α4 and β5 elements of the core structure. An ~22Å long tunnel leads from the surface of bMGL to the catalytic triad and thus keeps the active center unexposed to the polar environment. The rim at the entrance to the tunnel has a diameter that is ~12Å wide and is lined with hydrophobic side chains mostly from the cap domain. A hole is located in the cap region of the protein and corresponds to the proposed exit hole for glycerol to leave the active site in human MGL. Molecular dynamics simulation shows open and closed states of the entrance tunnel and the glycerol exit hole.

No electron density was observed for a stretch of 3 aa in the cap region. In human MGL, a corresponding region was also found to be highly flexible and was not visible in one chain of the dimeric structure. Comparison of the cap regions of hMGL and bMGL shows intriguing similarities, despite a sequence identity of only 10%. The 62-amino acid cap of hMGL has been described as U-shaped and comprises of three helices connected by loop regions. The 45-amino acid cap of bMGL harbors a short helical turn and a short two-stranded antiparallel β-sheet connected by linkers. It is very interesting that despite these differences in length and secondary structure composition, the overall shape and arrangement of the cap region of both the proteins superimposes almost precisely in the observed open conformation. It can be speculated that this specific lid architecture is conserved throughout evolution of MGLs.

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Keywords: lipase, crystallography, hydrolase

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A hybrid multifunctional antioxidant system: peroxiredoxin nitroreductase enzyme

Alda Navaza,[a] Pascalita Prosper,[b] Jean-Pierre Jacquot,[c] Nicolas Rouhier,[c] Ahmed Haouz,[d] [a]*UFR SMBH, Université Paris13 (France).* [b]*UMR, CRM2 UHP Nancy 1.* [c]*UMR 1136 INRA/UHP Nancy 1, UFR STB (France).* [d]*PF6, Institut Pasteur, Paris (France).*E-mail: alda.navaza@univ-paris13.fr

A natural fusion occurring between a peroxiredoxin (Prx) and a nitroreductase (Ntr) motif has been identified in *Thermotoga maritima*. The corresponding protein TmPrxNtr, the mutant TmPrxNtrC40S and its separate modules (TmPrx, TmNtr) have been overexpressed in *Escherichia coli,* then purified and their catalytic activity studied. TmPrxNtr and TmPrxNtrC40S display similar circular dichroism spectra showing that the mutation of cysteine 40 into serine does not