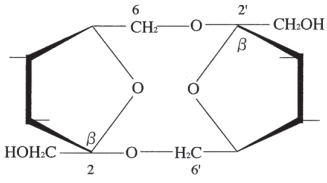
fructose, with a terminal glucose residue, and is found in some plants and bacteria. This polysaccharide serves as storage carbohydrates, and has shown to play a role in a tolerance for drought and low temperature in plants. In some microorganisms, levan is enzymatically degraded into several forms of 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

MS93.P40

Acta Cryst. (2011) A**67,** C782

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

Acta Cryst. (2011) A**67,** C782-C783

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)