present in microorganisms, plants and animals. From a biotechnological point of view, *Kluyveromyces lactis* β-galactosidase is suitable for many applications due to its neutral optimum pH and for the fact that *K. lactis* is a GRAS organism (Generally Recognized As Safe). Interestingly, β-galactosidases are being used in lactose intolerance treatments and in food industry. Moreover, yeast expressing this enzyme can be used to improve the valorization of the cheese whey, a cheese industry byproduct by coupling the degradation of lactose with ethanol production, biomass production, etc. [1].

On the basis of their sequence, β-galactosidases are classified within families 1, 2, 35 and 42 of glycosyl hydrolases in the CAZy database [2]. Those from eukaryotic organisms are grouped into family 35 with the only exceptions of *K. lactis* and *K. marxianus* β-galactosidases which belong to family 2, together with the prokaryotic β-galactosidases from *Escherichia coli* and *Arthrobacter* sp. Whereas the structures of these last two prokaryotic enzymes have been determined [3], [4], none of the eukaryotic β-galactosidase structures have been reported to date. Although their sequence similarity with the prokaryotic enzymes is significant (48% vs. *E. coli* and 47% vs. *Arthrobacter*) there are many differences, particularly some long insertions and deletions, which play an important role in protein stability and in substrate recognition and specificity.

Gaining insight into the structural features that determine its stability and understanding the specificity determinants and catalytic mechanism should lead to improvements of its biotechnological applications by rational protein engineering. In this study, we describe X-ray crystallographic studies and an analysis of *Kluyveromyces lactis* β-galactosidase structure.

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Keywords: yeast, glycosidase, biotechnology

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Structural studies of β–D-xylosidase from *Streptomyces thermoviolaceus* OPC-520

Koji Tomoo,[a] Kei Saito,[a] Tsunego Usui,[a] Toshimasa Ishida,[a] Katsushiro Miyamoto,[b] Hiroshi Tsujibo,[b] Raita Hirose,[c] Kensaku Hamada,[c] Yuki Nakamura,[d] Go Ueno,[e] Masaki Yamamoto,[e] [a]*Department of Physical Chemistry and* [b]*Department of Microbiology, Osaka University of Pharmaceutical Sciences, (Japan).* [c]*X-ray Research Laboratory, Rigaku Corporation, (Japan).* [d]*Rigaku Corporation, (Japan).* [e]*SR Life Science Instrumentation Unit, RIKEN SPring-8 Center. (Japan).* E-mail: tomoo@gly.oups.ac.jp.

Xylan is one of the major hemicellulose components of plant cells and is the second most abundant resource after cellulose. *Streptomyces thermoviolaceus* OPC-520 produces relatively high xylanase activity when grown in a medium containing xylan as a carbon source.

An intracellular β–D-xylosidase (BxlA) from *Streptomyces thermoviolaceus* OPC-520, together with a extra-cellular BxlE and the integral membrane proteins BxlF and BxlG, constitutes a xylanolytic system that participates in the intracellular transport of xylan degradation products and the production of xylose. To elucidate the hydrolytic mechanism of xylooligosaccarides to xylose at the atomic level, we attempted X-ray structural analysis of BxlA. The recombinant BxlA protein (molecular weight: 82kDa) was crystallized by the hanging-drop vapor-diffusion method at 293 K. The crystals belonged to the monoclinic space group *C*2, with unit-cell parameters *a*=141.0, *b*=129.5, *c*=100.4 Å, β=120.1°, and contained two molecules per asymmetric unit (V_M=2.47 Å^3/Da). Diffraction data were collected to a resolution to 2.2 Å on the BL26B1 beam line at the SPring-8. The brilliant light source present at SPring-8 was necessary to obtain useful data from these very weakly diffracting crystals. The initial structure of BxlA was determined by MAD method using the SeMet-labeled crystal. The structure of BxlA was built from typical catalytic domain and additional c-terminal domain. We now progress the refinement of structure to elucidate the detailed mechanism of glycoside hydrolysis of BxlA at the atomic level.

Keywords: xylosidase, MAD phasing, crystallographic structure

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Structural study of the mutants of FDTS from *T. maritima*

Irimpan I. Mathews,[a] Todd Fleischmann,[b] Amnon Kohen,[b] and Scott A. Lesley,[c] [a]*2575 Sand Hill Road, Stanford Synchrotron Radiation Lightsource, SLAC, Stanford University, Menlo Park, California 94025, (USA).* [b]*Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, (USA).* [c]*The Joint Center for Structural Genomics at the Genomics Institue of Novartis Research Foundation, San Diego, California 92121, (USA).* E-mail: iimathew@slac.stanford.edu

Like thymidylate synthase (TS) in eukaryotes, the flavin-dependent thymidylate synthase (FDTS) is essential for cell survival of many prokaryotes for the *de novo* synthesis of thymidylate (dTMP). TS and FDTS inhibition stops DNA production, eventually leading to cell death. The two enzyme families present no structural or sequence homology. While both convert dUMP to dTMP, in contrast to the classical TS enzymes, the FDTS enzymes employ 5,10-methylene-5,6,7,8-tetrahydrofolate (CH2H4folate) as the methyl donor but not as reducing agent. Furthermore, recent studies showed that, in contrast to TS, FDTS doesn't use enzymatic nucleophile for the reaction and that the catalytic mechanism of TSs and FDTSs is substantially different [1]. These differences offer the possibility of developing specific FDTS inhibitors as antibiotic drugs with low toxicity.

We report the structural study of five mutants of FDTS from *Thermotoga maritima*. All of the mutants crystallize in the orthorhombic space group $P2_12_12_1$ with unit cell dimensions of a=~55Å, b= ~116Å, and c=~141Å. A comparison of the structures with the wild type enzyme and a comparison of the structures of the mutant enzymes with and without the substrate dUMP show several features that are important for the FDTS reaction. The structural information is also compared with the activity data to get a better understanding of the reaction mechanism.

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Keywords: flavin, thymidylate, synthase

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Structure determination of levan fructotransferase

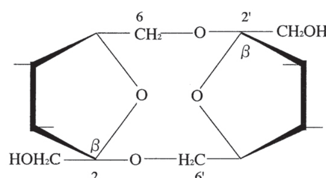
Jinseo Park, Myung-Il Kim, Sangkee Rhee, *Departament of Agricultural Biotechnology, Seoul National University, Seoul 151-921, (Korea).* E-mail: sun44@snu.ac.kr

Levan is fructan composed of linear and branched polymers of

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

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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)