Molecular and Cellular Biosciences, The University of Tokyo. [d]*RIKEN Harima Institute at SPring-8.* [e]*Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo.* E-mail: gon@tsurumi.yokohama-cu.ac.jp

Precursors for isoprenoid synthesis are essential in all organisms. These compounds are synthesized by one of two known routes: the well characterized mevalonate pathway [1] or a recently discovered non-mevalonate route which is used in many bacteria and human pathogens [2]. Since the second pathway is both vital and unlike any found in humans, enzymes catalysing reactions along this synthetic route are possible drug targets. The structure of one such enzyme from the thermophilic bacterium *Thermus thermophilus* has been solved to high resolution in the presence of substrate and with a substrate analogue. Enzyme co-crystallized with substrate shows only one product, cytosine monophosphate (CMP), in the active site. At the high resolution of the refinement (1.6 Å) the positions and coordination of the magnesium ions in the active site are clearly seen.

[1] Qureshi N., Porter J. W., *Biosynthesis of IsoprenoidCompounds*, J. W. Porter & S. L. Spurgeon, John Wiley New York, 1981, **1**, 47-94. [2] Rohmer M., Knani M., Simonin P., Sutter B., SahmH., *Biochem. J.* 1993, **295**, 517.

Keywords: isoprenoid synthesis, non-mevalonate route, drug targets

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Crystal Structure of the β-subunit of Acetyl-CoA Carboxylase in *C. glutamicum*

Ryo Natsume[a], Minoru Yamada[a,b], Miki Senda[a], Tsuyoshi Nakamatsu[b], Sueharu Horinouchi[c], Hisashi Kawasaki[b], Toshiya Senda[d], [a]*JBIRC, JBIC.* [b]*Materials Sci. Eng., Grad. Sch. Eng., Tokyo Denki Univ.* [c]*Dept. of Biotech., Grad. Sch. of Agriculture and Life Sciences, the Univ. of Tokyo.* [d]*BIRC, AIST.* E-mail: rnatsume@jbirc.aist.go.jp

Acetyl-CoA carboxylases (ACCs) catalyze the first committed step of fatty acid biosynthesis. Although ACC is an essential enzyme (complex) in every organism, the structure-function relationship of ACC remains to be unclear. As the first step for elucidating the structure-function relationship of ACC, we started the crystallographic analysis of DtsR1. DtsR1 is the β-subunit of ACC multisubunit complex in *Corynebacterium glutamicum*, which catalyzes the transcarboxylation between biotin and acetyl-CoA.

DtsR1 was over-expressed in *E. coli*, purified, and crystallized by the sitting-drop vapor diffusion method using PEG 6000 as a precipitant. The approximate dimensions of the obtained crystals were 0.07x0.07x0.03mm^3. Diffraction data of the crystals were collected at NW12 of the Photon Factory (Tsukuba), revealing that the crystals belong to the space group *R*32. The crystal structure of DtsR1 was solved at 3.2Å resolution by the molecular replacement method using single-subunit coordinates of the 12S transcarboxylase (PDB ID: 1ON3) as a search model. The obtained structure suggests that the biological unit of DtsR1 is a ring-shaped hexamer with the *32*-point group symmetry. Crystallographic refinement of DtsR1 is in progress at 2.7Å resolution.

Keywords: carboxylases, fatty acid biosynthesis, structure-function enzymes

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Structural Studies of FlaA1, a UDP-GlcNAc 4,6-dehydratase

Noboru Ishiyama[1], Melinda Demendi[2], Carole Creuzenet[2], Joseph S. Lam[3], Albert M. Berghuis[1,4], *Departments of* [1]*Biochemistry and* [4]*Microbiology & Immunology, McGill University, Montreal, Quebec H3A 2B4, Canada.* [2]*Department of Microbiology & Immunology, University of Western Ontario, London, Ontario N6A 5C1, Canada.* [3]*Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada.* E-mail: noboru.ishiyama@mail.mcgill.ca

FlaA1 is a UDP-GlcNAc 4,6-dehydratase believed to be involved in the protein glycosylation process of *Helicobacter pylori*. The crystal structures of FlaA1 in five different ternary complexes with various substrates were determined at resolutions between 1.9 and 2.8 Å. This represents the first structure of a 4,6-dehydratase that can catalyze a UDP-saccharide. Among 4,6-dehydratases, FlaA1 possesses several unique structural features including a novel C-terminal fold and a hexameric oligomerization state in the crystal. The catalytically productive conformation observed in the FlaA1•NADPH•UDP-GlcNAc ternary complex suggests that FlaA1 employs a different mechanism for the water elimination step from that proposed for other 4,6-dehydratases. Normally, an Asp and Glu residues are the two catalytic residues that effect dehydratase activity through a concerted mechanism. In FlaA1, the corresponding residues are Asp-132 and Lys-133, precluding an analogous mechanism. Computational analysis suggests that for the water elimination step in FlaA1, Lys-133 sequentially functions as catalytic acid and base while Asp-132 closely interacts with the leaving water group.

Keywords: dehydratase, catalytic mechanism, pKa calculation

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The Crystal Structure of Murine 11β-hydroxysteroid Dehydrogenase 1: an Important Therapeutic Target for Diabetes

Jiandong Zhang, Timothy D. Osslund, Matthew H. Plant, Christi L. Clogston, Rebecca E. Nybo, Fei Xiong, John M. Delaney, Steven R. Jordan, *Amgen Inc., Amgen Center Drive, Thousand Oaks, CA 91320, USA.* E-mail: zhang@amgen.com

11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyzes the conversion of 11-dehydrocorticosterone to its active form corticosterone in rodents (or cortisone to cortisol in humans). An excess of active glucocorticoids has been shown to play a key role in metabolic disorders such as diabetes and obesity. Therefore, 11β-HSD1 represents an important therapeutic target for the treatment of these diseases. To facilitate the iterative design of inhibitors, we have crystallized and determined the three-dimensional structures of a binary complex of murine 11β-HSD1 with NADP(H) to a resolution of 2.3 Å, and a ternary complex with corticosterone and NADP(H) to a resolution of 3.0 Å by X-ray crystallography. The enzyme forms a homodimer in the crystal. The structure shows a novel folding feature at the C-terminus of the enzyme. The C-terminal helix insertions provide additional dimer contacts, exert an influence on the conformations of the substrate binding loops, and present hydrophobic regions for potential membrane attachment. The structure also reveals how the 11β-HSD1 achieves its selectivity for its substrate.

Keywords: 11β-HSD1, SDR, corticosterone

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Crystal Structure of P-protein of the Glycine Cleavage System

Tadashi Nakai[ab], Noriko Nakagawa[ab], Nobuko Maoka[a], Ryoji Masui[ab], Seiki Kuramitsu[ab], Nobuo Kamiya[a], [a]*RIKEN Harima Institute at SPring-8, Hyogo, Japan.* [b]*Graduate School of Science, Osaka University, Osaka, Japan.* E-mail: nakaix@spring8.or.jp

The glycine cleavage system (GCS) is a multienzyme complex composed of four different components (P-, H-, T- and L-proteins). In almost all organisms, the GCS plays a crucial role in the degradation of glycine, and it has been studied extensively. Three-dimensional structures of H-, T- and L-proteins from many species have been published, but only the structure of the P-protein has not yet been reported. We have determined the crystal structure of the P-protein from *Thermus thermophilus* HB8, which reveals that P-proteins do not involve the α_2-type active dimer universally observed in the evolutionarily related pyridoxal 5'-phosphate (PLP)-dependent enzymes. Instead, novel αβ-type dimers associate to form an $\alpha_2\beta_2$ tetramer, where the α- and β-subunits are structurally similar and appear to have arisen by gene duplication and subsequent divergence with a loss of one active site. The binding of PLP to the apoenzyme induces large open–closed conformational changes. The structure of the complex formed by the holoenzyme bound to an inhibitor, (aminooxy)acetate, suggests residues that may be responsible for substrate recognition. The molecular surface around the lipoamide-