Biosci. Biotech., Tokyo Institute of Tech., Japan. E-mail: kaoru@iwakimu.ac.jp

Carbonic anhydrase (CA) is a zinc-containing enzyme that catalyzes the reversible hydration of CO_2. Based on the amino acid sequences, CAs are classified into three evolutionarily distinct families, designated α-, β- and γ-CAs. CA from *Chlamydomonas reinhardtii* belong to the α type enzyme and, homologous to CA from animal and eubacteria source. To reveal the structure, we have been engaged on X-ray study of *Chlamydomonas* CA. The enzyme was extracted from Cultured *Chlamydomonas* cells and highly purified by column chromatography [1]. The enzyme is composed of two subunits, small and large. Crystals were obtained in a solution containing ammonium sulfate. X-ray diffraction data were collected at 100K with synchrotron radiation at NW12 of PF-AR (Tsukuba). Diffraction patterns were processed with the program HKL2000. The crystal diffracted to a maximum resolution of 2.4Å. The unit-cell dimensions were a=b=134.6 and c=120.0Å with a space group of $P6_1$ or $P6_5$. Initial phases were derived by the molecular replacement method using the atomic coordinates of CA from *Neisseria gonorrhoeae*, which has a sequence identity of 37 % with the present CA.

[1] Yang S-Y., Tsuzuki M., Miyachi S., *Plant Cell Physiol*.,1985, **26**, 25-34.

Keywords: carbonic anhydrase, *Chlamydomonas reinhardtii*, X-ray crystal structure analysis

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Crystallographic Study of Fructokinase from *Sulfolobus tokodaii* strain7

Takeshi Sekiguchi[a], Shintaro Ishiguro[a], Isao Koike[b], Yohei Maeda[b], Kaoru Suzuki[a], Akio Takenaka[b], [a]*College of Scinece and Engineering, Iwaki-Meisei University.* [b]*Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan*. E-mail: sekiguch@iwakimu.ac.jp

Fructokinase (FK) (ATP:D-fructose-6-phosphotransferase, EC 2.7.1.4) catalyses the phosphorylation of fructose-6-phosphate with ATP. This enzyme is essential for removing fructose to prevent the reverse reaction, in glycolytic pathway. The crystal structures of FK were reported only for prokaryote, *Bacillus subtilis* and *Salmonella enterica*. To reveal the tertiary structure of FK from an Archaea *Sulfolobus tokodaii* strain7, we have performed X-ray analyses of the protein. *Sulfolobus* FK produced in *E. coli* was purified by column chromatography, and crystallized. X-Ray diffraction data taken at 100K show that there are two forms with $P2_12_12_1$ and $P6_122$. Their initial crystal structures were derived by molecular replacement with 2-keto-3-deoxygluconate kinase from *Thermus thermophilus*, and refined to 2.8Å and 1.85Å resolutions, respectively. The two tertiary structures are similar to each other, but quite different from that of *Bacillus* FK with a little bit high sequence identity (29%). The present structures are rather similar to *Salmonella* FK though the sequence identity is low at 27%. An interesting feature is that although the function is different from *Thermus* 2-keto-3-deoxygluconate kinase, but the two tertiary structures are similar at higher sequence identity (35%).

Keywords: fructokinase, *Sulfolobus tokodaii* strain7, X-ray crystal structure analysis

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Crystal Structure of Translation Initiation Factor IF2beta-IF2gamma Complex

Min Yao, S. Toya, N. Sakai, M. Sokabe, I. Tanaka, *Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, Japan.* E-mail: yao@castor.sci.hokudai.ac.jp

IF2 is a heterotrimeric GTPase composed of alpha, beta, and gamma subunit. It has been identified in both of eukaryotes and archaea. The gamma subunit is core subunit which binds Met-$tRNA_i^{Met}$ and GTP. The beta subunit was reported to interact with eIF5 that stimulates GTP hydrolysis, and also interact with Met-$tRNA_i^{Met}$ and mRNA [1,2], while alpha subunit functions as a regulatory element of IF2.

We have determined the structures of aIF2beta-aIF2gamma and aIF2beta-aIF2gamma-GDP complex

The aIF2beta subunit was located at G-domain of aIF2gamma and was close to GTP-binding site. The detail of the complex structure will be discussed and the model of ternary complex IF2-GDP-Met-$tRNA_i^{Met}$ will be given in this presentation.

[1] Das S., Maiti T., Das K., Maitra U., *J. Biol. Chem.*, 1997, **272**, 31712. [2] Laurino J. P., Thompson G. M., Pacheco E., Castilho B. A., *Mol. Cell. Biol.*, 1999, **19**, 173.

Keywords: translation, translation initiation factor, IF2

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Fusidic Acid Resistance and Sensitivity in Ribosomal Elongation Factor G

Sebastian Hansson[a], Ranvir Singh[a], Anatoly Gudkov[b], Anders Liljas[a], Derek Logan[a], [a]*Molecular Biophysics, Lund University, Sweden.* [b]*Institute of Protein Research, Russian Academy of Science, Pushchino, Moscow region.* E-mail: Sebastian.Hansson@mbfys.lu.se

Elongation factor G (EF-G) catalyzes translocation in protein synthesis on the ribosome. EF-G is inhibited by Fusidic acid (FA), a commonly used antibiotic. Structural information on FA binding to EF-G has not yet been available.

We present three crystal structures of two mutant EF-G factors; G16V, highly FA sensitive, and T84A, highly FA resistant. The crystal structures provide a first insight into the conformational changes induced by GTP binding and how this affects FA binding. These structural conformations show the general importance of the interface of domain G, III and V as a key component of the FA binding site and the specific role of Phe90 as a gatekeeper and conformational regulator. We provide an explanation on how EF-G is able to discriminate between GDP and GTP.

Keywords: protein synthesis, translation factors, antibiotic resistance

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The *thrS* Messenger Path on the Ribosome

Lasse Jenner[a], Pascale Romby[b], Bernard Rees[a], Dino Moras[a], Gulnara Yusupova[a], Marat Yusupov[a], [a]*Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France.* [b]*Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France.* E-mail: lasse@igbmc.u-strasbg.fr

Initiation of translation generally determines the efficiency of protein synthesis, and is the key step for the control of gene expression when fast adaptation is required. Binding of the messenger to the eubacterial ribosome involves several mRNA elements, which affect the kinetics of the initiation complex formation. These elements may include binding sites for translational regulatory proteins. *E. coli* threonyl-tRNA synthetase (ThrRS) plays such a role, as it inhibits its own synthesis by binding to *thrS* mRNA in a region located upstream from the ribosome binding site.

Here we show the path of *thrS* mRNA on the ribosome in the presence of the initiator $tRNA^{Met}$ at 5.5 Å of resolution determined by X-ray crystallography. Our data show the first visualization of a translational regulatory domain of mRNA and explain how a repressor protein can interfere with initiation of ribosome translation.

Keywords: ribosome structure and function, tRNA synthetases, regulation

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Productive and Non-productive Binding of Polyketides to the Ribosome Large Subunit

Rita Berisio[a], Maya Amit[b], David Baram[b], Joerg Harms[c], Anat Bashan[b], Harly Hansen[c], Frank Schluenzen[c], Ada Yonath[b], [a]*IBB,*