Takeshi Sekiguchi[3]
[1]Tokyo Institute of Technology, Graduate School of Bioscience and Biotechnology, 4259 Nagatsuda, Midori-ku,, Yokohama, Kanagawa, 226-8501, Japan, [2]Faculty of Pharmacy, Iwaki-Meisei University, 970-8551 Iwaki, Japan, [3]College of Science and Engineering, Iwaki-Meisei University, 970-8551 Iwaki, Japan, [4]IGBMC, 67404 Illkirch, France, E-mail：atakenak@bio.titech.ac.jp

To maintain high fidelity in translation, twenty kinds of aminoacyl-tRNA synthetases (ARSs) exist in general for twenty kinds of amino acids, each ARS being specialized to recognize only the cognate amino acid (A) and the cognate $tRNA^A$. Some organisms, however, have two genes for ThrRS, and considered that their proteins (ThrRS-1 and ThrRS-2) are complementary to each other in functions, one for catalysis and the other for editing. In order to clarify their three-dimensional structures, we started X-ray analyses of putatively assigned ThrRS-1 (APE0809) and ThrRS-2 (APE0117) from *Aeropyrum pernix* (*Ap*), and those (ST0966 and ST2187) from *Sulfolobus tokodaii* (*St*). These proteins were overexpressed in *E. coli*, purified, and crystallized. The crystal structure of *Ap*-ThrRS-1 has been successfully determined at 2.3 Å resolution, as the first example. *Ap*-ThrRS-1 is a dimeric enzyme composed of the two identical subunits, each containing two domains for the catalytic reaction and for the anticodon-binding. The essential editing domain is, however, completely missing as expected. These structural features are consistent with that ThrRS-1 catalyze only the aminoacylation of the cognate tRNA, and suggest the necessity of the second enzyme ThrRS-2 for editing. Since the N-terminal sequence of *Ap*-ThrRS-2 is similar to the sequence of the editing domain of ThrRS from *Pyrococcus abyssi*, *Ap*-ThrRS-2 is expected to catalyze de-aminoacylation of the misacylated serine moiety at the CCA terminus.

Keywords: aminoacyl-tRNA synthetase, crenarchaea, aeropyrum pernix

P04.09.259

Acta Cryst. (2008). A**64**, C312

The structure of archaeal ribosomal stalk complex

Isao Tanaka[1], Takao Naganuma[1,2], Min Yao[1], Toshio Uchiumi[2]
[1]Hokkaido University, Faculty of Advanced Life Science, Kita-ku, Kita-10, Nishi-8, Sapporo, Hokkaido, 060-0810, Japan, [2]2 Department of Biology, Faculty of Science, Niigata University, Niigata, 950-2181, Japan, E-mail：tanaka@castor.sci.hokudai.ac.jp

Ribosome has a highly flexible lateral protuberance called the stalk at the GTPase-associated center on the large subunits. This ribosomal stalk is universally conserved in all domains of life and plays a crucial role for replenishing translation factors which catalyze translation reaction in a GTP-dependent fashion. The detailed studies of the archaeal ribosomal stalk complex of *Ph*P0(*Ph*L12)$_2$(*Ph*L12)$_2$(*Ph*L12)$_2$ from the hyperthermophilic archaean *Pyrococcus horikoshii* showed that it has the ability to access to eukaryotic elongation factors at the levels comparable to that of eukaryotic stalk (Nomura, 2006). Therefore, the archaeal heptameric stalk complex and the eukaryotic pentameric stalk complex apparently share conserved functional structures. In order to understand detailed structural and functional characteristics of archaeal ribosomal stalk complex, we solved the crystal structure of the archaeal ribosomal stalk complex from *P. horikoshii* at 2.3 Å resolution by Se-MAD method. This archaeal ribosomal stalk complex is composed of *Ph*P0(*Ph(*L12)$_2$(*Ph*L12)$_2$(*Ph*L12)$_2$ whose flexible C-terminal parts are truncated. Comparing with bacterial ribosomal stalk complex L10(L12)$_2$(L12)$_2$(L12)$_2$, the *Ph*P0 has longer and more flexible C-terminal helical spine composed of three helices with linker loops (helix-loop-helix-loop-helix). The manner by which the L12 dimers bind to the C-terminal spine are also very different.

References
[1] Nomura, T., Nakano, K., Maki Y., Naganuma T., Nakashima,T., Tanaka,I., Kimura, M., Hachimori, A., Uchiumi, T. (2006). *Biochemical J.* **396**, 565-571.

Keywords: structures of macromolecules, ribosomes, translation factors

P04.10.260

Acta Cryst. (2008). A**64**, C312

Successful cryocooling of protein microcrystalline samples for powder diffraction

Yves Watier[1], Irene Margiolaki[1], Jonathan Wright[1], Andrew Fitch[1], Mathias Norrman[2], Gerd Schluckebier[2]
[1]ESRF, Material science ID31, 6 rue jules horowitz, Grenoble, Isere, 38000, France, [2]Novo Nordisk A/S, Copenhagen, E-mail：watier@esrf.fr

Modern developments of the powder diffraction technique have allowed the investigation of systems with large unit cells such as proteins [1]. Protein powder specimens consist of a large number of randomly oriented diffracting micro-crystals. These micro-crystals are usually formed rapidly by batch crystallization. Frequently, the resolution and quality of the data are limited mainly by rapid deterioration of the protein crystal structure during exposure to the intense synchrotron X-ray beam. In a typical single crystal diffraction experiment radiation damage can be minimized by collecting diffraction data under cryocooled conditions (typically 100 K) which requires the addition of a cryoprotecting agent to the protein sample to prevent freezing of the mother liquor. In this study, we succeeded in obtaining various cryocooled samples of human insulin at 100 K avoiding ice formation. Powder diffraction data were collected at both room temperature and under cryocooled conditions (ID31, ESRF, Grenoble, France). As expected both the cryoprotectant and the sample container have a remarkable impact on the data quality. Significant variation of the lattice parameters and peak widths with the type and concentration of cryoprotecting agent has already been observed and will be presented for the case of insulin. Preliminary data interpretation correlating these changes with the structural and microstructural characteristics of the systems under study will be shown.

[1] Margiolaki, I. & Wright, J. P. *Acta Cryst*. (2008). **A64**, 169-180

Keywords: protein, powder diffraction, cryocooling

P04.10.261

Acta Cryst. (2008). A**64**, C312−313

New development of frozen buffer-free crystal mounting method for the longer wavelength SAD phasing

Nobuhisa Watanabe[1,2], Yu Kitago[2], Isao Tanaka[2]
[1]Nagoya University, Synchrotron Radiaction Research Center, Furo-cho, Chikusa-ku, Nagoya, Aichi, 464-8603, Japan, [2]Hokkaido University, N10 W8 Kita-ku, Sapporo, Hokkaido, 060-0810, Japan, E-mail：nobuhisa@nagoya-u.jp

Using longer wavelength X-ray for the SAD phasing is one of the trends in macromolecular crystallography. At longer wavelength, the anomalous intensity difference or the Bijvoet ratio of almost all sulfur containing protein crystals is around 1% of total reflection intensity.

This Bijvoet difference, however, is still small and highly accurate data collection is essential. One of the experimental difficulties using longer wavelength is the increased absorption. Therefore we have developed a crystal mounting technique to eliminate absorption by the frozen cryo-buffer around protein crystal (Kitago et al., 2005), and the practical applicability of this mounting method was examined using several novel proteins at CrKα radiation of 2.29 Å (Watanabe, 2006). In order to utilize this mounting method at the synchrotron beamlines, we made this mounting tool compatible to the standard Hampton CrystalCap. With a special magnet base for this cap, it becomes possible to mount and remount frozen crystals as the standard CrystalCap. We have tested its applicability by sending several frozen crystals to the beamline BL13B1 at NSRRC, Taiwan. This mounting method is also very useful at synchrotron beamlines to mount tiny crystals that are difficult to center because of the lens-shaped frozen buffer in the cryoloop. In this development, we also use a loop made of a polyimide film microfabricated by photolithography.

[1] Kitago, Y., Watanabe, N. and Tanaka, I. (2005), *Acta Cryst*., **D61**(8), 1013-1021.
[2] Watanabe, N. (2006), *Acta Cryst*., **D62**(8), 891-896.

Keywords: SAD, longer wavelength SAD, crystal mounting method

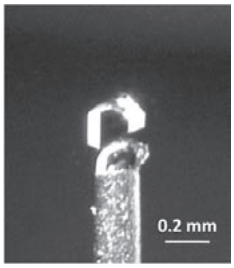
P04.10.262

Acta Cryst. (2008). A**64**, C313

Application of a novel mounting tool using adhesive for protein crystals

Tomoya Kitatani[1,5], Tomokazu Hasegawa[2], Sugiyama Shigeru[1,5], Hamada Kensaku[2], Matsumura Hiroyoshi[1,4,5], Takano Kazufumi[1,4,5], Adachi Hiroaki[1,4,5], Murakami Satoshi[3,4,5], Inoue Tsuyoshi[1,4,5], Mori Yusuke[1,4,5]
[1]Osaka University, Department of Electrical Engineering, Graduate School of Engineering, 2-1 Yamadaoka, Suita, Osaka, 565-0871, Japan, [2]PharmAxess, Inc., Osaka 567-0085, Japan, [3]The Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567-0047, Japan, [4]SOSHO Inc., Osaka 541-0053, Japan, [5]CREST JST, Suita, Osaka 565-0871, Japan, E-mail：kitatani@chem.eng.osaka-u.ac.jp

We have developed a novel mounting tool for protein crystals that directly captures a crystal from a drop with an adhesive (Kitatani *et al*., 2008). This tool has a loop-free structure and can reduce the amount of solution around the captured crystal in comparison with conventional loop-based mount tools. The extra solution around captured crystal not only degrades the quality of data-collection statistics but also disturbs the initial phase determination by the sulfur single-wavelength anomalous diffraction (SAD) method. The effect of reducing the solution in this tool is helpful in the sulfur SAD method. We have evaluated the effects of our tool on a quality and crystallographic data in the sulfur SAD method using CoK_α radiation.



Reference
[1] Kitatani T., Sugiyama S., Matsumura H., Adachi H., H. Yoshikawa Y., Maki S., Murakami S., Inoue T., Mori Y. and Takano K. (2008) *Appl. Phys. Express*, 1, 037002.

Keywords: biomaterials and biodevices, protein crystals, SAD

P04.10.263

Acta Cryst. (2008). A**64**, C313

Structural transitions and the evolution of protein crystal disorder during slow cooling

Matt A Warkentin[1], Robert E Thorne[1,2]
[1]Cornell University, 219 Clark Hall, Ithaca, NY, 14853, USA, [2]Mitegen LLC, Ithaca, NY, 14850, USA, E-mail：maw64@cornell.edu

We report measurements of the lattice perfection and unit cell volume of Lysozyme, Thaumatin, and Trypsin crystals as function of temperature during slow cooling (200 K/hour) from 300 K to 100 K. We demonstrate that these crystals can be slow cooled without formation of internal crystalline ice, even when no penetrating cryoprotectants are used. Most degradation of diffraction properties occurs between 240 and 180 K. In Thaumatin crystals, slow cooling produces low temperature diffraction properties that are as good as or better than those obtained with flash cooling or with cooling under pressure. In Lysozyme and Trypsin crystals, crystal order abruptly and dramatically degrades near 210 K. This dramatic degradation is associated with an apparent first-order phase transition signaled by an abrupt decrease in unit cell volume. We examine two possible origins for this transition: cold denaturation of the protein, and a transformation between low density and high density liquid water within the nanometer-sized channels of the crystal.

Keywords: cryocooled crystallography, structural phase transitions, biological macromolecular crystallography

P04.10.264

Acta Cryst. (2008). A**64**, C313

The temperature dependence of radiation damage to macromolecular crystals

Robert E Thorne[1,2], Matthew A Warkentin[1]
[1]Cornell University, Physics Department, 219 Clark Hall, Ithaca, NY, 14853, USA, [2]Mitegen LLC, Ithaca, NY, 14850, USA, E-mail：ret6@cornell.edu

We have studied how the rate of global radiation damage to Lysozyme, Thaumatin, and Trypsin crystals varies with temperature. The radiation sensitivity of these proteins decreases by a factor of 30 to 50 between T=300 K and 100 K. Most of the decrease occurs between 220 K and 180 K, and we attribute this to a glass transition in the protein + solvent system. Addition of 40% glycerol raises the temperature for the drop in radiation sensitivity by ~10 K, consistent with the measured shift in the glass transition.

Keywords: radiation damage, biological macromolecular crystallography, glass transition

P04.10.265

Acta Cryst. (2008). A**64**, C313-314

Manipulating protein microcrystal with optical tweezers based on lensed fiber probes

Takaaki Hikima, Tetsuya Shimizu, Masaki Yamamoto
RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo, 6795148, Japan, E-mail：hikima@spring8.or.jp

In many synchrotron facilities, X-ray microbeam will be utilized for protein crystallography. It will be possible to collect diffraction data from a protein microcrystal with the size in the range from 1