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

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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
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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.

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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.