three specific uridine bases in *Escherichia coli* ribosomal 23S RNA to pseudouridine: bases 955, 2504, and 2580 in the case of RluC and 1911, 1915, and 1917 in the case of RluD. Both have an N-terminal S4 RNA binding domain. While the loss of RluC has little phenotypic effect, loss of RluD results in a much reduced growth rate. We have determined the crystal structures of the catalytic domain of RluC, and full-length RluD. The S4 domain of RluD appears to be highly flexible or unfolded and is completely invisible in the electron density map. Despite the conserved topology shared by the two proteins, the surface shape and charge distribution are very different. The models suggest significant differences in substrate binding by different pseudouridine synthases. [1]

[1] Mizutani K., Machida Y., Unzai S., Park S.-Y., Tame J.R.H., *Biochemistry*, 2004, **43**, 4454-4463.

Keywords: RNA-binding proteins, structure, protein crystallography

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Crystal Structure of PilF from *Pseudomonas aeruginosa*

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The tetratrico peptide repeat (TPR) is a structural motif present in a wide range of proteins. It mediates protein-protein interactions and the assembly of multiprotein complexes. TPR motifs have been identified in various different organisms, ranging from bacteria to humans. Proteins containing TPRs are involved in a variety of biological processes, such as cell cycle regulation, transcriptional control, mitochondrial and peroxisomal protein transport, neurogenesis and protein folding. Type IV pilus biogenesis protein, PilF of *Pseudomonas aeruginosa* consists of 253 amino acids and makes up 3 tandem TPR motifs. It is known to require for correct fimbrial biogenesis. We could express the PilF of *Pseudomonas aeruginosa* in an *E.coli* expression system and produced selenomethionie-substituted crystal, which diffract to 2.5 Å. It belongs to P222 space group and unit cell is a=68.4 Å, b=70.0 Å, c=138.1 Å. This structure of the full sized TPR protein will lead to the first step in study of TPR interaction.

[1] Stover C.K., Pham X.Q., *Nature*, 2000, **406**, 31. [2] Watson A.A., Alm R.A., *Gene*, 1996, **180**, 49.

Keywords: PilF, TPR domain, crystal structure

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Crystallization and Data Collection of *Xanthomonas citri* Maltose-Binding Protein

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In this work we report the crystallization and analysis of prelliminary data of the periplasmic maltose-binding protein (MBP) of the plant pathogen *Xanthomonas citri*, responsible for the canker disease affecting citrus plants all over the world. The 50,1 kDa protein has been overproduced in *Escherichia coli*, purified, and crystallized in complex with its substrate maltose. The crystallization of MBP using the sitting-drop vapour-diffusion method with PEG 20000 as precipitant is described. Crystals belong to the orthorhombic space group P2(1)2(1)2(1), with unit-cell parameters a = 105,83, b = 105,21, c = 262,32 Å. X-ray diffraction data were collected to a maximum resolution of 3.2 Å using a synchrotron-radiation source. Structure refinement is in progress.

Structural analysis, in combination with ongoing biochemical characterization, will assist the elucidation of the structure-activity relationship in regulating the uptake of maltose in this bacteria.

Keywords: MBP, *Xanthomonas citri*, crystallization

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Crystal Structure of Ubiquitin-like Domain of Murine Parkin

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Parkin, which has been identified ubiquitin ligase, is the gene product of autosomal recessive juvenile parkinsonism (AR-JP). Parkin which consists of 464 amino acid residues has three domains; an N-terminal ubiquitin-like domain(ULD) and two RING finger-like domains. Parkin has important role in recognition of the target proteins and addition of the ubiquitin in proteasome system. In order to elucidate the fully function of Parkin, we have started the structure analysis of Uld of murine Parkin.

The recombinant murine Uld was expressed as inclusion body from *E.coli.* system. After refolding and purification, we crystallized Uld by hanging-drop vapor diffusion method. Under the condition of 0.1M acetate buffer(pH4.5) and 3M NaCl as a precipitant. The crystal belong to the hexagonal system, with unit cell dimensions of a=b=45.57 Å, c=64.75 Å, γ=120°. Diffraction data were collected up to 1.8 Å resolution at beam line BL24XU of SPring-8. The initial structure was determined by molecular replacement by using the solution structure of Uld as start model. Refinement of structure is currently in progress.

Keywords: ubiquitin system, crystallization, structure analysis

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SERCA1a and Phospholamban Cocrystallisation

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The Sarco(Endo)plasmic Reticulum Ca^{2+}-ATPase (SERCA) is a membrane Ca^{2+}-pump with a crucial role in the relaxation/contraction mechanism of the muscular cells.

SERCA1a has been purified from Sarcoplasmic Reticulum vesicles, isolated from rabbit fast twitch muscles. Ca^{2+}-ATPase concentration was increased within SR vesicles using different techniques: high ionic strength was employed to eliminate myosin and many membrane proteins and vesicles were treated with EDTA with the same purpose. Furthermore SR membranes have been purified by an extraction with low concentration of deoxycholate. Purified membranes were solubilised using a non-ionic detergent, $C_{12}E_8$, at 1.8 mg/ml final concentration. The supernatant was directly used for crystallization. Crystals of E1 SERCA1a grew in few days at 19°C with the hanging drop technique, using a precipitant solution containing: 15% (w/v) PEG 6000, 4% (v/v) tert-butanol, 15% (v/v) glycerol, 5 mM β-mercaptoethanol, 200 mM sodium acetate [1].

Synthesized PLB was solubilised in a solution containing chloroform/methanol with a ratio of 1/2 to a 31 mg/ml final concentration. SERCA1a and PLB were mixed to a 1:5 final molar ratio. Cocrystals grew in approximately a week, using the same precipitant utilized in SERCA1a crystallization.

[1] Sorensen T.L., Moller J.V., Nissen P., *Science*, 2004, **304**, 1672.

Keywords: SERCA1a, phospholamban, cocrystallisation

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Structural Studies on Collagen binding Integrin αI Domains

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Integrins are a large family of cell adhesion receptors that mediate