different from that of other nuclear receptors.

Keywords: nuclear receptor, Fushi tarazu factor 1, FTZ-F1

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X-ray diffuse scattering from protein crystals caused by the lattice defects

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High resolution X-ray protein crystallography needs a single crystal of high quality. The quality has been often described with a mosaicity. However, the intrinsic nature of the quality of protein crystal has not yet been understood well. Phenomenologically speaking, a crystal of poor quality causes the decrease of the Bragg reflection intensity and does not give higher order Bragg reflections. It has been developed to estimate the quality of proteins by measuring the B-factor.1) The B-factor consists of static and dynamic components and the quality of protein crystals may correspond to the orientation disorder of molecules in the crystal. Therefore the disorder structure will be determined by analyzing X-ray diffuse scattering on the foot of the Bragg reflections. We have carried out the measurement of the X-ray diffuse scattering from a cubic insulin crystal which has given medium resolution data (2.2Å). The size of the sample is about $0.3\text{mm} \times 0.3\text{mm} \times 0.3\text{mm}$. We have used 4-circle diffractometer installed at BL10A in Photon Factory in KEK, Japan. The beam divergence is 1.23×10^{-6}[rad]. We have measured several rocking curves of Bragg reflections of [100], [110] and [111] series at the ambient temperature and succeeded in observing the diffuse scattering on the foot of these Bragg reflections. In order to make the origin of the diffuse scattering clear, we are planning to measure several crystals which have grown under different crystallization condition and have different qualities.

1) S.Arai, T.Chatake, N.Suzuki, H.Mizuno and N.Niimura: Acta Cryst. D60, 1032-1039 (2004)

Keywords: static and dynamic disorder, quality of protein crystals, diffuse scattering

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Structure of membrane-bound quiohemoprotein alcohol dehydrogenase

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Many Gram-negative aerobic bacteria can grow on alcohols and sugars as the sole carbon and energy sources. In the periplasm of acetic acid bacteria, quinoprotein alcohol dehydrogenases (ADH) containing pyrroloquinoline quinine (PQQ) instead of nicotinamide or compounds as the prosthetic group catalyze the first step of acetic acid production, oxidation of ethanol to acetaldehyde. There are three types of ADHs. Type I ADH is a soluble, dimeric protein of identical subunits having a PQQ and a calcium ion in each active center, but no other redox cofactors. Type II ADH is a soluble, monomeric, having a PQQ-containing catalytic domain and an additional *c* domain with a covalently bound heme *c*. Type III ADH is a quinohemoprotein complex with three nonidentical subunits that catalyzes the oxidation of ethanol and the subsequent reduction of ubiquinone, and attached on the cytoplasmic membrane of acetic acid bacteria. We report here 3.0 Å crystal structure of the type III membrane-bound quinohemoprotein ADH from *Gluconobacter suboxydans* refined to *R*-factor 29 %. Our structure reveals that the enzyme contains a large subunit A similar to the type II quinoprotein ADHs which have a eight-stranded propeller domain and a cytochrome *c* domain, a membrane-bound subunit B which has a novel three-heme cytochrome *c* structure, and a small subunit C which has unknown function. The PQQ is located near the axis of the propeller domain about 14 Å from the in subunit A. The shortest distances between four hemes are about 9 Å, 4 Å, and 8 Å, respectively.

Keywords: crystal structure analysis, membrane protein structures, heme proteins

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Rational crystallization of β-lactoglobulin and vitamin D_3 complex reveal a secondary binding site

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β-lactoglobulin (β-LG) is a major bovine milk protein with a predominantly β structure. The function of the only α-helix with three turns at the C-terminus is unknown. Vitamin D binds to the central calyx formed by the β-strands. Despite being one of the most investigated proteins whether there are two vitamin D binding-sites in each β-LG molecule has been a subject of controversy during the past forty years. In this study, we chose vitamin D3, instead of vitamin D2, and use rational approach to successfully form a β-LG-vitamin D3 complex for crystallization. The only difference of vitamin D3 from D2 is the latter being a double bond between the carbon positions 22 and 23. Vitamin D3 is well-fitted into the bulk of electron density at 2.4 Å-resolution around the calyx and the exosite. In the central calyx binding mode, the aliphatic tail of vitamin D3 clearly inserts into the binding cavity, where the 3-OH group of vitamin D3 binds externally. The electron density map suggests that the 3-OH group interacts with the carbonyl of Lys-60 forming a hydrogen bond. The second binding site, however, is near the surface at the C-terminus containing part of an α-helix and a β-strand I with 17.91 Å in length, while the span of vitamin D3 is about 12.51 Å. A remarkable feature of the second exosite is that it combines an amphipathic α-helix providing non-polar residues and a β-strand providing a non-polar and a buried polar residue. They are linked by a hydrophobic loop. Thus, the binding pocket furnishes strong hydrophobic force to stabilize vitamin D3 binding. This finding provides a new insight into the interaction between vitamin D3 and β-LG, in which the exosite may provide

another route for the transport of vitamin D3 in vitamin D3 fortified dairy products.

Keywords: beta-lactoglobulin, vitamin D3, protein complex crystallization

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Large single crystal growth and preliminary neutron diffraction analysis of *Achromobacter* protease I

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Achromobacter protease I (API, E.C. 3.4.21.50) is one of the serine proteases produced by *Achromobacter lyticus* M497-1. API is distinct from the trypsin type serine protease in its lysine specificity, a higher peptidase activity, pH optimum raging from pH 8.5-10.5 and stability against denaturation with urea and SDS, respectively. Due to these favorable properties as a protein-degrading enzyme, API is useful as the lysylendopeptidase for protein fragmentation and lysyl bond formation. From the X-ray structure analysis of API, several hydrogen bonds play an important role and one water molecule is located at an active site of this protein. To elucidate these results in detail by observing protons, hydrogen bonds and hydration structure of the protein, we have carried out neutron diffraction experiment. Neutron crystallography, however, needs a large single crystal because intensity of neutron beam is still limited. Large crystals which are applicable to neutron diffraction experiment were grown on the basis of the crystallization phase diagram. A crystal of API grew up to 2.0mm x 1.0mm x 0.5mm by vapor diffusion method with modified macroseeding procedure. Crystals were soaked in 50%PEG3350/D_2O. The D_2O exchanged structure was determined by x-ray diffraction experiment to obtain an initial model for neutron structure analysis. Neutron diffraction data were collected with BIX-4 installed at the JRR-3 of the Japan Atomic Energy Agency (JAEA). Neutron structure analysis is currently underway by using CNS 1.1 as a refinement software.

Keywords: neutron crystallography, protease, large single crystal

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Purification and crystallization of a C-terminal domain of a human single-pass transmembrane protein

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To identify novel factors involved in mRNA transport from the nucleus, eleven novel temperature-sensitive mutants (*ptr1-11*), which accumulate poly(A)$^+$ RNA in the nuclei at the nonpermissive temperature in *Schizosaccaromyces pombe* were isolated. Ptr10p, one of these causative gene products, is a single-pass transmembrane protein localized in nuclei and endoplasmic reticulum. The location of the DnaJ domain at the N-terminal region suggests that Ptr10p may interact with Hsp70 through its DnaJ domain and play a role in mRNA export from the nucleus. On the other hand, the function of the C-terminal domain of Ptr10p cannot be predicted from its amino acid sequence because it shares little sequence homology with known proteins. In order to reveal the structural and functional insights into the C-terminal domain of Ptr10p, we have purified and crystallized the C-terminal domain of human Ptr10p (hPtr10p-C, 111 amino acids), which shows 29 % amino acid sequence identity with *S. pombe* Ptr10p. hPtr10p-C was expressed as a N-terminal GST fusion protein in *E. coli* and purified by GST affinity chromatography followed by thrombin digestion to remove the GST-tag, and cation-exchange chromatography. We obtained high-purity hPtr10p-C, but it is liable to aggregate during concentration for crystallization. We therefore optimized salt and detergent concentrations of the protein solution, and the purified protein solution was successfully concentrated to 18 mg ml^{-1} suitable for crystallization trials. The initial crystallization screening of hPtr10p-C was carried out by the hanging-drop vapor diffusion method. We succeeded to obtain crystals from a condition containing 2-propanol as a precipitant, and the crystal diffracted to better than 3 Å on an in-house X-ray source.

Keywords: purification, crystallization, mRNA transport

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Crystallization and preliminary X-ray analysis of RNA aptamer in complex with human immunoglobulin G

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Aptamers are short DNA or RNA folded molecules that can be selected in vitro on the basis of their high affinity for a target molecule. An optimized 23-nucleotide aptamer was prepared, and was shown to bind to the Fc domain of human IgG, but not to other IgG's, with high affinity. To obtain a more detailed insight into the molecular mechanism of RNA aptamer to recognize - and bind to - human IgG with high specificity and affinity, we have initiated a crystallographic study of RNA aptamer in complex with human IgG. Initial crystals of the RNA aptamer- human IgG complex were grown by the vapor-diffusion method. But polycrystals appeared within two weeks and were not of sufficient quality to diffract X-rays. After optimization of the crystallization condition, suitable crystals were obtained by combining the shaking and sitting-drop vapor-diffusion methods. We will report a comparative study of shaking-grown and traditional grown crystals of the RNA aptamer- human IgG complex.

Keywords: RNA-protein interactions, protein crystallization, X-ray diffraction