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