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 ranging from pH 8.5-10, and stability against denaturation with urea and SDS, respectively. Due to these favorable properties as a protein-degrading enzyme, API is useful as the lysyldendopeptidase 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 macroseedling procedure. Crystals were soaked in 50%PEG3350/D2O. The D:O 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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**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 sitting-drop grown crystals of the RNA aptamer-human IgG complex.

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