High Resolution and Zn MAD experiment of CD13 Protein

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There are two types of coronavirus surface receptors. The groups II coronavirus mouse hepatitis virus uses murine carcinoembryonic antigen-related cell adhesion molecules as receptor. While group I viruses, for examples human 229E, transmissible gastrioentreritis virus and feline infectious peritonitis virus, use CD13 or APN as receptor for cell entry. Recently a distinct coronavirus has been identified as the agent of SARS. This make the studies of receptors of coronavirus become important.

CD13, also known as aminopeptidase N (APN), belongs to family of membrane peptidases and is a multifunctional ectoenzyme. It has been implicated in the control of growth and differentiation of many cellular systems. Human CD13 gene is located at 15q25-q26 and its size is 3.5kb. It contains 20 exons and belongs to gluzincins superfamily, which comprised HELAH motif of Zn2+-binding site. CD13 is anchored to the cell membrane by a transmembrane helical region near the N-terminus, with only a small region of the N-terminus (8-10 amino acids) protruding into the cytoplasm. In most species CD13 exists as a homodimer. It widespreads in many cells and its main sources being liver, brush borders of kidney, small intestine and placenta. In the myelomonocytic lineage, CD13 is found on precursors, monocytes, basophils, eosinophils and neutrophils. Its activity can be inhibited by actininin, EDTA, amastatin, probestin, o-phenanthroline and bestatin. The dual regulatory aspects of membrane peptidases-being regulated by cell-cell contacts themselves as well as influencing cellular functions play important roles in cellular function and cell growth.

Angiogenesis, the formation of new blood vessels, is a rate-limiting step in solid tumor growth. Angiogenic blood vessels express markers, such as integrins, that are at abnormal high level in tumor cells. There are three peptide motifs, RGD, NGR and GSL, capable of homing to tumor vasculature. Coupling an antitumor drug to one of these motifs yielded compounds with increased efficacies against tumors and lower toxicity to normal tissues. APN (CD13) has been shown to be the receptor of NGR motif and vascular structures with detectable APN are tumor blood vessels. APN antagonists are antiangiogensis *in vivo*. These findings indicate that APN plays a functional role in angiogenesis. In addition, APN also involved in cell mobility and APN expression may be useful indicator of a poor prognosis for node-positive patients with colon cancer.

The monoclonal antibodies (mAbs) to APN/CD13 can evok the rise in $[Ca^{2+}]_i$ in monocytes. Tyrosine kinase inhibitors were able to inhibit the rise in $[Ca^{2+}]_i$ induced by ligation APN/CD13, as were inhibitors of the phosphatidylinositol 3-kinase. These findings show that mAbs to APN/CD13 provoke phosphorylation of the mitogen-activated protein kinases ERK1/2, JNK, and p38. Furthermore, mRNA of the chemotactic cytokine IL-8 is upregulated under the influence of APN/CD13 ligation. Although the *in vivo* ligand as well as possible cooperating membrane molecules remains to be identified, the membrane ectoenzyme APN/CD13 has been shown to be a novel signal transduction molecule in monocytes.

Since highly glycosylation of APN/CD13 it is very difficult to crystallize this protein. However, we have very small crystals of APN. The small size of these crystals make this project only doable in synchrotron and either Zn MAD or Sulfur SAD are the only possibility to solve the structure of this protein. There are two Zn ions and 20 Met residues and 2 Cys residues in CD13 protein. Its structure can be solved by Zn MAD w/o Sulfur SAD experiment. We proposed a High resolution data collection of native protein and a Zn MAD experiment for this protein. This is the reason why we need synchrotron experiment.

In this cycle (Nov23-25) we have 6 shifts of beam time. we carried another 20 crystals and they are new crystal form of thioesterase complex with octanoic acid and BBA and human serum albumin complex with drugs. We did collect 5 sets of good data sets for thioesterase crystals up to 1.6 A resolution. One Mandelate racemase soaked with Hg crystal was used to collect a MAD data set. 13 albumin crystals were screened and only two crystals were used to collect data. However, these crystals all undergo a phase transition, diffraction spots from two phases are obtained from the same crystal, which is difficult to resolve..

Proposal Title: X-ray Crystallographic Study of Novel Protein Folds and Virulence Factors of *Helicobacter pylori*

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Helicobacter pylori, a small Gram-negative bacterium, colonizes in approximately half of human population, induces chronic inflammation in gastric epithelial cells, and may further develop into various gastrointestinal diseases such us peptic ulcers and gastric cancer. Despite the hypothesis of the altered physiology of the stomach arising from both bacterium as well as high polymorphism of host, the molecular mechanism underlying the host-bacterium remains largely unknown. Given the availability of the whole genome and new approaches including structural genomics and proteomics, it is rational to investigate *H. pylori* molecular pathogenesis by those new emerging forces. For solving the crystal structures, we need to use different wavelengths to obtain the MAD data and the powerful synchrotron source will also help us to obtain high resolution data that we can get more detail of our structures.

In this study in SPring-8 beamline 12B2, several crystals of HP0283 proteins were collected using synchrotron radiation source. Several high resolution data sets including native data sets were collected. The crystals diffract to the resolution of about 2.0 Å with the mosaicity of 0.35 and the overall R_{merge} below 10%. The structure is going to be solved by using SHELX or SHARP method. The crystals of HP0283 protein MAD data were collected that diffract to the resolution of about 2.0 Å and the over all R_{merge} below 10%. The HP1249 crystals diffract well to the resolution of about 2.2Å with the mosaicity of 0.25 and the overall R_{merge} below 10%. The structure is going to be solved by using MR method.

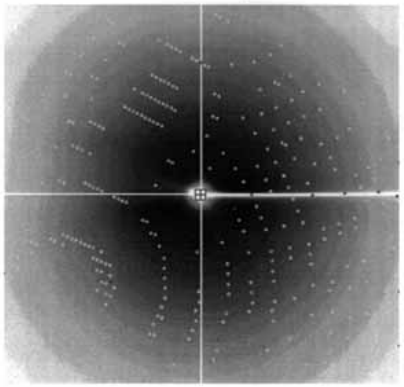

Figure 1: diffraction pattern of HP0283 native crystal with 1.0degree collected

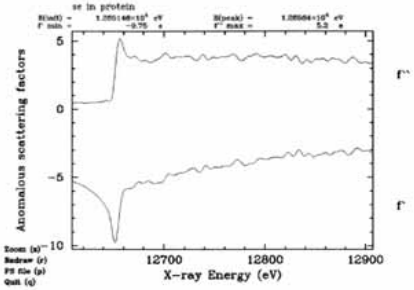

Figure 2: The X-ray scattering anomalous (HP0283).