High Resolution and Zn MAD experiment of CD13 Protein

Yen-Chywan Liaw*(5116), Shan-Sheng Huang(9598), Fang-Ju Tsai(9111), Imameddin(9000) and Yun-Wen Chen(9126)
Institute of Molecular Biology, Academia Sinica, Taiwan, R.O.C.

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 the cycle (Dec 14-Dec 15) we planed to collect Zn-MAD data sets. However, just before we came to spring-8, we had a power failure for our cold room and all the crystals die. We only had two crystals left. After we came here we found these two crystals decay to almost dead. In the cycle (Feb 14-Feb16) we planed to collect Zn-MAD data sets. Before we went to Spring-8 we check the crystals and we only get about 10A resolution. We hope to get improved data from Spring-8. However, we get about the same resolution in Spring-8 by unknown reason, which is too low to do MAD experiments. However, we also carried another 10 crystals and they are thioesterase complex with BBA. After screening these crystals, we did collect 2 sets of good data sets for thioesterase crystals up to 2.3 A resolution. The first one is coded thio8m with 5.3% of Rmerge, 99.1% of completeness, 67% of last shell of data greater than 2 I/sigmal, 17.8 of redundancy, 18.9 of average I/sigmal. The second one is coded thio9m with 6.5% of Rmerge, 98.7% of completeness, 70% of last shell of data greater than 2 I/sigmal, 21.6 of redundancy, 17.1 of average I/sigmal. Using molecular replacement we solved the structure and refined on both data sets. Current R factors are 21.85 and 22.68, respectively, and Rfree are 26.04 and 28.48. The interesting point we found from current map is that BBA is not covalently linked to serine oxygen. This is very interesting and consistent with data observed from NMR studies. We might see an intermediate state of reaction. Detail analysis and refinement is still under going.

Structural Determination of Colicin E7 Translocation Domain by MAD

Wei-Jen Yang (4447), Po-Chao Wen (14014), Lyudmila Doudeva, Che-Chuan Yang (14007), Pan-Hsien (14008) and Hanna S. Yuan * (4583)
Institute of Molecular Biology, Academia Sinica, Taiwan, ROC.

Colicins are *Escherichia coli* secreted toxins which are expressed to kill other closely related bacteria cells in stressful conditions. ColE7 is one of the nuclease-type colicins containing a non-specific endonuclease domain capable of hydrolyzing DNA in target bacterial cells. The N-terminal domain of ColE7 contains a receptor-binding and a membrane translocation domain. In order for ColE7 protein to enter the cytoplasm of susceptible cells, the ColE7 have to interact with the outer membrane BtuB receptor by its receptor-binding domain and traverse the membranes by the help of its membrane translocation domain.

Colicin-producing bacteria are resistant against the action of their own colicin through possession of a small immunity protein that inactivates the cytotoxic domain. After binding to *E.coli* cell surface receptors, E-type colicins are translocated to their site of action by a *tol*-dependent translocation system. The 3.0 Å X-ray structure of the RNase colicin E3 has been determined. The Translocation domain of E3 forms a jellyroll structure composed of the three _ sheets flanked by two helical stretches but the first 83 residues are disordered, as there is no electron density corresponding to this region. There are 34 glycine residues within the first 79 residues, which corresponds to a glycine content of 43%. In spite of its apparent flexibility, this N-terminal region contains the sequence recognition motif for binding to TolB.

The Se-labeled translocation domain of ColE7 was crystallized and the diffraction data at the Se absorption edge were collected up to 2.7 Å in BL12B. Some of the crystals were able to diffract up to ~1.6 Å, thus, more detail structural information for the translocation domain of colicin family protein is expected to be obtained once the high resolution crystal structure is solved.

The translocation domain of ColE7 has a molecular weight of ~30 KD. Se-Met labeled translocation domain of ColE7 was crystallized by the hanging-drop vapor diffusion method. About 7 data sets were collected in 3 wavelengths (E_{peak}, E_{rem} and E_{infl}) using Se-Met labeled crystals. The statistics for one of the data set are listed below.

Data collection statistics

Cell constant	a = 58.0 Å, b = 58.0 Å, c = 130 Å α = β = 90°, γ = 120°
Space Group	P321
Wave length E_{peak}	0.979749 Å
E_{rem}	0.964077 Å
E_{infl}	0.979965 Å
Unique reflections	7,024
Reflections collected	37,075
Completeness ~2.7 Å	92.3%
$R_{sym\ linear}$	9.0 %