Proposal Number [C04B12B2-1011N] **BL12B2**

Structural genomic studies of Helicobacter Pylori by X-ray crystallography

Jia-Yin Tsai(9573), Po-Kai Lu(13998), Hsiu-Chien Chan(15280), and Yuh-Ju Sun(8457)
Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Hsinchu 300, Taiwan, ROC.

Helicobacter pylori is a gram-negative bacterium, which colonize the gastric mucosa of humans, causing gastritis and peptic ulcer disease, and is associated with certain types of gastric cancers. *H. pylori* has well-developed systems for motility, for scavenging iron, and for DNA restriction and modification. Many putative adhesions, lipoproteins, and other outer membrane proteins were identified, underscoring the potential complexity of the host-pathogen interaction. In 1994, the International Agency for Cancer Research declared that *H. pylori* were a carcinogen of humans. However, we do not yet clearly understand the transmission of this bacterium.

Here, we choose the complete genome sequences of *Helicobacter pylori* strains 26695 as the target genome to do the structural proteomics and functional genomics study. For the phase determination, the hypothetic protein Se_Met mutated HP0367 was cloned, purified, and crystallized. The Se-Met mutated HP0367 protein was collected data at BL12B2 Taiwan beamline at Spring-8, Japan. The three wavelengths (0.97964Å, 0.97994Å, and 0.9626Å) were chosen for the data collection of Se_Met HP0242 crystal. It can diffract to 3Å resolution. We collected three data sets of HP crystal. The phase determination was using SOLVE/RESOLVE programs.

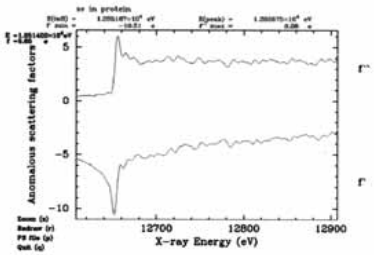

Figure 1. The graph of energy versus absorption for the SE-Met mutated HP0367protein.

Table 1. X-ray diffraction statistical data of Se-Met mutated HP0367protein.

Crystal	SeMet		
Data Collection			
Source	Spring-8 BL12B2		
Wavelength (Å)	0.97964	0.97994	0.9626
f'	6.08	2.24	3.41
f''	4.88	-10.51	-3.23
Crystal system	Hexagonal	Hexagonal	Hexagonal
Space group	$P6_522$	$P6_522$	$P6_522$
Unit cell parameters			
a (Å)	125.7	125.9	125.8
b (Å)	125.7	125.9	125.8
c (Å)	68.5	68.6	68.5
Resolution (Å)	3.0	3.0	3.0
Number of reflections collected	72932	71618	77764
Number of unique reflections	6089	5975	6479
Redundancy of reflection	12.0	12	12
Completeness (%) overall (outmost shell)	95.2(100)	94(100)	95.6(99.2)
I/σ(I) overall (outmost shell)	23.5(9.6)	28.5(11.1)	23.2(10.3)
R_{merge}* (%) overall (outmost shell)	8.7(30.2)	7.3(27.3)	8.9(39.1)
Mosacity(°)	0.272	0.295	0.276
χ^2	1.032	1.018	1.024

*$R_{merge}(I) = \sum_h \sum_i |I_i - \bar{I}| / \sum_h \sum_i I$ where I is the mean intensity of the i observation of reflection h.
†Data processed by HKL2000.

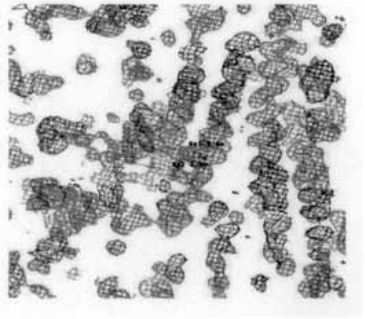

Figure 2. The preliminary phase of the SE-Met mutated HP367 protein.

Proposal Number [C04B12B2-1012N] **BL12B2**

Crystal structure of PriB-dT15mer complex

Yi-Hung Yeh(008453), Che-Hsiung Hsu(014121), Kuang-Lay Tsai(015613), Chwan-Deng Hsiao(006899)
Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan, R.O.C.

Analyses of primosome assembly at chromosomal and plasmid origins as well as that at single-stranded replication origins revealed the presence of two distinct primosomes in *Escherichia coli* for primer RNA synthesis and duplex unwinding. A DnaA-dependent primosome is assembled at ***ori*C**, the chromosomal origin of *Escherichia coli*, as well as at the A site. In contrast, PriA protein recognizes a hairpin, called PAS (primosome assembly site), and initiates assembly of the **ΦX174**-type PriA-dependent primosome in conjunction with other prepriming proteins.

Seven primosomal protein, PriA, PriB, PriC, DnaB, DnaC, DnaG and DnaT, are required for the assembly of a primosome at the primosome assembly site (PAS) on a single-stranded DNA-binding protein (SSB)-coated **ΦX174** phage DNA. PriB stabilizes PriA on the DNA, this cannot be the sole reason it facilitates binding of DnaT, and it is likely that PriB-induced conformational rearrangements contribute as well. EMSA data appear that dT ssDNA binding capacity is better than dA ssDNA, so we complexed PriB with dT15mer and crystallize this complex. We want to understand PriB how to interact with ssDNA.

We got many thin crystals and resolution is only 4Å. The crystal of PriB-dT15mer belong to the Monoclinic space group $P2_1$ and cell parameter is a=47.4Å，b=45.67Å，c= 51.48Å，β=96.1°. There are two PriB molecules per one asymmetric unit. We solved the phase problem by using MR method from PriB model. The structure of PriB is similar to that of single-stranded DNA binging protein (SSB), even though they share only 16% amino acid sequence identity.

But PriB-dT15mer complex diffraction data is only 4Å by in-house light source. We would like to get high resolution PriB-dT15mer diffraction data by synchrotron light source in Spring-8.

Result :

We collect two crystal forms diffraction data, one is $P2_1$ and it cell parameter is a=47.4Å，b=45.67Å，c= 51.48Å，β=96.1°, resolution is about 2.8Å and the other is P222 and its cell parameter is a=47.4 Å，b=45.67 Å，c= 101.48 Å, resolution is about 3.75Å. we get high resolution diffraction data from SPring-8.