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Structural genomic studies of Helicobacter Pylori by X-ray crystallography

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Helicobacter pylori is a spiral-shaped, Gram-negative microorganism that was found in 1979 and isolated in 1982. It is one of the most successful human bacterial parasites, colonizes more than half of the human population, and is identified as an etiologic agent in a variety of gastroduodenal diseases. 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. Two complete genome sequences of *Helicobacter pylori* have been determined by the whole-genome random sequencing method. One is *Helicobacter pylori*, strain 26695 (cagA+ /vacA+), has a circular genome of 1,667,867 base pairs and 1,590 predicted coding sequences and the other is *Helicobacter pylori*, strain J99 (cagA+ /vacA+), isolated in the USA in 1994 from a patient with a duodenal ulcer, has a circular genome of 1,643,831 base pairs and 1,495 predicted coding sequences.

We choose the complete genome sequences of *Helicobacter pylori* strains 26695 as the target genome to do the structural proteomics and functional genomics studies. The hypothetic protein HP0242 was cloned, purified, and crystallized. For the phase determination, the Se-Met mutated HP0242 protein was also prepared and crystallized. The Se-Met mutated HP0242 crystal belongs to the orthorhombic crystal system with $P2_12_12_1$ space group. The X-ray diffraction data was collected at BL12B2 Taiwan beamline in Spring-8, Japan. The four wavelengths (0.9795Å, 0.9648Å, and 0.9800Å) were chosen for the data collection of Se-Met HP0242 crystal. It can diffract to 2.5Å resolution. The data was processed and analyzed by HKL2000. The X-ray diffraction data statistics of the SE-Met mutated HP0242 crystal are listed in table 1. The V_M of HP0242 crystal was calculated to be 1.94 $ÅDa^{-1}$ and the solvent content was estimate to be 37%. The structure determination is in process.

Figure 1. The graph of energy versus absorption of selenium for the SE-Met mutated HP0242 crystal.

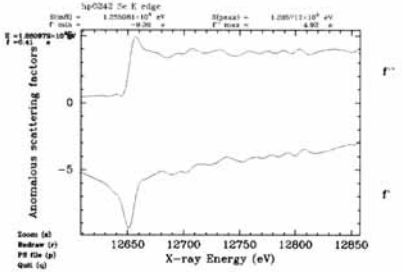

Table 1. Summary of X-ray diffraction data statistics of the SE-Met mutated HP0242 crystal.

Wavelength	0.9795 Å (150 frames)	0.9800 Å (150 frames)	0.9648 Å (180 frames)
Scattering factors (e)	f'' = 4.9e f' = -7.1e	f'' = 2.6e f' = -9.4e	f'' = 3.83e f' = -3.1e
Unit Cell a b c	42.25 Å, 70.87 Å, 116.08 Å	42.34 Å, 70.99 Å 116.19 Å	42.33 Å 70.98 Å 116.19 Å
Total #of Reflections	73496	73738	88595
# of Unique Reflections	12612	12638	12721
Redundancy	5.83	5.83	6.96
Completeness* (_)	99.9/99.4	99.9/99.4	99.9/99.4
I/σ*	18.5/4.8	18.2/3.0	20.8/4.78
R_{merge} (_)*	8.0/38.9	8.9/62.5	8.2/44.1
Mosacity (°)	0.82	0.87	0.83
χ^2	1.049	1.083	1.065

*:(average/outer shell)

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Crystallographic studies of Interleukin enhancer binding factor 1 and PriB complexes with DNA

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Interleukin enhancer binding factors (ILFs) are trabscription factors and bind to purine-rich regulatory motifs in both human T-cell leukemia virus long terminal region (HIV V-1 LTR) and the interleukin-2 (IL-2) promoter. Specifically, ILFs bind to the antigen receptor response elements 2 (ARRE-2) of IL-2 promoter. Although the function of ILFs is not well understood, previous role in regulating IL-2 gene expression. To date, three ILFs have been found: ILF-1, ILF-2, and ILF-3 with 655, 609, and 323 amino acids, respectively. Both ILF-1 and ILF-2 contain several amino acid homologies including a region for potential ubiquitin-mediated degradation, a nuclear localization signal, an N-glycosylation motif, and a DNA-binding domain. The DNA-binding domain belongs to the winged helix family since residues from 251 to 348 of ILF1 and ILF-2 share 35 to 88% similarity with other known members of this family.

We had collected high resolution data, but we can't use molecular replacement to solve phase problem. In order to investigate the 3-D structure of the ILF proteins, we grew the crystals of Se-Met mutanted ILF proteins, and collected data in BL12B2, Spring-8, Japan.

The 4 wavelengths (0.964311Å, 0.979551 Å, 0.979989 Å, and 0.987796 Å) were chosen for the data collection of ILF crystal. It can diffract to 3.5 Å resolution. We collected four data sets of ILF crystal. But this protein's Se-Mets are all in disorder region, so we try the Shelx and Solve program, we can't search correct phase.

Bacterial chromosomal DNA replication requires assembly of a primosome responsible for primer synthesis and continuous unwinding of double-stranded DNA at the replication fork. In *E. coli*, there are at least two types of distinct primosomes that have been identified *in vitro*. One that is assembled at oriC is dependent on Dna A protein, and the other that depends on Pri A protein is assembled at n'-pas sequence. The in vitro reconstitution of both primosomes has been reported. In Pri A-dependent primosomal proteins remains uncertain. Recently, we discovered that transfection with plasmid containing wild-type pri B gene enabled the rescue of the M4 *E.coli*, a ts mutant encoding the permuted PriB (F77A), from cessation of DNA replication at unpermissive temperature. These laboratory findings suggest that Pri B protein is needed for DNA replication in vivo and imply the essential role of the Pri B protein for both Pri A- and Dna A-dependent pathways. The previously revealed Pri B activities *in vitro*, including the ability to interact with RNA, SSB-coated ssDNA, and the Pri A-DNA complex, besides being involved in the formation of primosome, a little light on its mechanism. However, the molecular mechanism of this important action of Pri B is not yet clear.

In order to investigate the 3D structure of the Pri B complexes with DNA, we grew the crystals of Pri B complexes with DNA crystal. This crystal can diffract to 3.5 Å by in house X-ray. So we expect that we will collect higher resolution data in BL12B2, Spring-8, Japan.

We collect higher resolution data in BL12B2, Spring-8, Japan. The resolution is about 2.8 Å, and use molecular replacement to search correct phase.

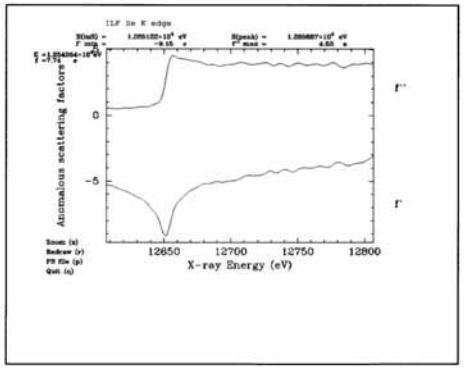

Fig. 1 The graph of energy versus absorption for the Se-Met mutanted ILF protein