1. Introduction

BL6B was constructed in 1995 with funds from 15 companies belonging to SBSP under provisions of a five-year agreement between TARA center in the University of Tsukuba and KEK, and opened to users in May 1996. SBSP moved from TARA to the Foundation for Advancement of International Sciences (FAIS) in April 2000. Maintenance fees for the BL6B station are supplied by SBSP. Beam-time is shared half and half by company users and normal PF co-users. A beam-time on-line booking and canceling system (1) where users can manage themselves was introduced subsequently, which allows urgent data collection, for example on short life crystals, and hence contributes to high throughput structure analysis. There have been more than 120 papers on protein crystal structure analysis as the basis of structural biology using BL6B in the last 4 years. All of this information is written both on the home page (1) and the Journal of the SBSP.

2. Performance of the BL6B station

a) Weissenberg data collection system (2)

BL6B is part of bending magnet beamline BL6, dedicated to data collection from protein crystals using monochromatic x-rays and a screenless Weissenberg camera with an IP cassette, into which either one or two large format (800mm x 400mm) imaging plates (IP) can be placed. The distance between sample crystal and the cassette is chosen to be either 575.3mm or 967.9mm, which enables us to collect data from very large unit cell crystals (~2000). An advantage of the Weissenberg camera is that more than ten times the amount of oscillation data can be obtained in an image compared the simple oscillation method, thus the Weissenberg camera is useful not only to speed up the data collection and data processing but also to get more accurate data, by not using partial reflections. The data collection system of BL6B consists of the Weissenberg camera, two IP-readers and a network (3) which connects the IP-readers, an Alpha Server-4000 in the SBSP container house, which is about 50m from the station, and computers operated by NIS and NFS to check and process the read out data. The network system (2) is high security and high speed and covers all protein stations,
that is, BL6A, BL6B, BL6C and BL18B. All data files on the data server are backed up once a day on DLTs and are kept at least half a year for the users. Three kinds of data processing software, Denzo (4), Process (5) and Weis (6) are provided. Various kinds of structure analysis software such as the MSI software package are also provided.

b) Comparison with similar beamline in other Institutes

There is no similar beamline, which has a screenless Weissenberg camera for protein data collection in the world except for UX4 1 at SPring-8. Since UX4 1 is an undulator beamline, intense and finely collimated x-rays are provided to the Weissenberg camera; on the other hand, the data collection system in UX4 1 does not connect a high speed and high security network with a large data server and many computers.

3. Scientific activities

a) Principal achievements

There are over 120 papers by members of SBSP and PF co-users. Since this beamline has been built by donations, we note here example papers from foreign and industrial users.

1) Three-dimensional structure and function of chromospheres in phycobiliprotein

The three-dimensional structure and function of chromospheres in phycobiliprotein has been clarified with this beamline. The light-harvesting system of algae is formed of phycobilisomes, which are composed of different phycobiliproteins; Phycoerythrin (PE), Phycocyanin (PC) and Allophycocyanin (APC). Light energy is harvested and transferred in the direction of the PE→PC→APC at photosynthetic reaction center, with very high efficiency. Phycobiliproteins all carry different chromospheres such as Phycoerythrobilin (FEB), Phycocynobilium Phycovilibilin and Phycourobilin. These chromospheres were arranged in a specific position and orientation in the protein to form a specific network for energy transfer. The energy transfer pathways inside each phycobiliprotein and phycobilisome, on the basis of three-dimensional structures at very high resolution, were proposed (7,8,9) as shown in Fig 1.
Fig. 1. Posturate model of energy transfer between two adjacent R-PE hexamer and chromophores a 140a and B 155

Fig. 2. Crystal structure of RNA dependent RNA polymerase Hepatise virus
Fig. 3. Crystal structure of cytochrome P450sca from Streptomyces carbophilus

2) Crystal structure analysis of the RNA dependent RNA polymerase of Hepatitis C virus (10)

The crystal structure of RNA dependent RNA polymerase of the Hepatitis C virus (HCV) was determined at 2.5Å resolution (Fig. 2). HCV is a positive single-stranded RNA [(+) sRNA] virus and the major etiological agent of hepatocellular carcinoma. According to a report from the World Health Organization, there are 170 million chronic HCV carriers in the world today. However, no vaccine is available and there is no broadly effective therapy for all genotypes of HCV. The NS5B protein of HCV is the RNA dependent RNA polymerase, which has the full functionality of HCV RNA genome replication, and is one of the potential targets for anti-HCV agents. The development of a drug is expected.

3) Crystal structure of cytochrome P450sca from Streptomyces cabophilus involved in production of pravastatin, a cholesterol-lowering drug (11)

Pravastatin, a drug for treating hypercholesterolemia, is produced by
Hydroxylation of ML-236B is catalyzed by only two components, P450sca and flavoprotein, and the P450sca system possesses novel characteristics in prokaryotes (12,13,14). Crystals of this protein have been obtained from ammonium sulfate solution and belong to P3121 with cell dimensions of a=103.5Å, c=79.8Å. The crystal structure was determined at 2.0Å resolution by using the SIR technique and was refined with 26959 reflections having F>30(F). The structure is shown in Fig. 3.

A substrate recognition region may exist in B,F and G helices in Fig. 3 from a comparison with those of P450cam (4). Crystal structure of novel acid phosphatase from E. blattae (15)

The structure of E. coli non-specific acid phosphatase has been determined at 1.9Å resolution (Fig. 4) and sulfite sites with high probability of binding with phosphate are shown in Fig. 5. This enzyme is a homohexamer of molecular mass 150kDa; EB-NSAP shares a conserved sequence motif not only with several lipid phosphatases.
Fig. 5. Stereo view of the active site structure. Hydrogen bonds are shown as dashed lines.

Fig. 6. Crystal structure of hTP0163/Fab complex (left) and hTP0163 region (right).
Fig. 7. Schematic model of RuvA tetramer-Holiday Junction complex and the mammalian glucose-6 phosphatases but also with the vanadium-containing chloroperoxidase (CPO) of Curvularia inaequalis. A comparison of the crystal structures of EB-NSAP and CPO reveals striking similarity in active site structures. In addition, the topology of the core of this enzyme shows considerable similarity to the fold of the active-site containing part of monomeric 67kDaCPO, despite the lack of further sequence identity. Further study of reaction mechanism will bring interesting and important findings in enzyme chemistry and applications to food science.

5) Crystal structure analysis of TPO/Fab complex (16)
Thrombopoietin (TPO) is a cytokine that specifically regulates megakaryocyte maturation and platelet production. Human TPO (hTPO) is a two-domain protein. The 153-residue N-terminal domain is homologous to four-helix bundle cytokines and is sufficient for full biological activity. The signal domain of hTPO (hTP0163) shown in Fig. 6 (right), which comprises the N-terminal 163 residues, crystallized effectively as a complex with Fab derived from the activity neutralizing mouse monoclonal antibody TN1. We solved the crystal structure of hTP0163/Fab complex shown in Fig. 6 (left) by molecular replacement using Fab coordinates at 2.9Å. TPO displays the same mix of long- and short-chain helical cytokine features as erythropoietin. The length and packing of the helices is representative.
of the long-chain family, such as growth hormone, while the short-chain family, such as interleukin.

6) Crystal structure of the RuvA tetramer-Holiday Junction complex

Homologous recombination is a crucial process of living cells. It is involved in rearrangement of genes within and between chromosomes and in damaged DNA repair. In eukaryotic cells, this process is also required for chromosomal segregation in mitosis. Holiday junction, in which DNA strands are exchanged between a pair of homologous DNA duplexes, is well known as a universal intermediate of genetic recombination. In the major pathway of homologous DNA recombination in prokaryotic cells, the Holliday Junction intermediate is processed through its association with RuvA, RuvB and Ruvc proteins. Specific binding of the RuvA tetramer to the Holliday junction is required for the RuvB motor protein to be loaded onto the junction DNA, and the RuvAB complex drives the ATP-dependent branch migration. We solved the crystal structure of a Holliday junction bound to a single E. coli RuvA tetramer at 3.1 Å resolution (Fig. 7). In this complex, one side of the DNA is accessible for cleavage by RuvC resolvase at the junction center. The refined junction DNA structure revealed an open concave architecture with four-fold symmetry. Each arm, with B-form DNA, in the Holliday junction is predominantly recognized in the minor groove through hydrogen bonds with two repeated helix-hairpin-helix motifs of each RuvA subunit. The local conformation near the crossover point, where two base pairs are disrupted, suggests a possible scheme for successive base pair rearrangements, which may account for smooth Holliday junction movement without segment unwinding.

4) Future plans; Production of valuable protein structures with high accuracy
The sequencing of the human genome finished before 2000 and that of other genomes also will finish during these five years. The new age called "post genome" will come very soon. According to Science News, amount of genomes sequenced in Japan is only 10%. People expect that more than 30% of three-dimensional structures of protein in structure genomics will be determined in Japan. In this period, the contribution of a data collection system using SR for structure determination must become very large and especially this beamline, constructed by industry and managed by the users themselves, will make a large contribution to the structural biology in the coming age. We hope many new valuable structures with high accuracy will be produced from this beamline during these two or three years. As the result of this, the fundamental base of Structural Biology of the SAKABE Project will be established and development to the next stage of the structural biology will be possible.

References