RIKEN Beamlines for Structural Biology

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1. Introduction

A knowledge of the three-dimensional structures of biological macromolecules is indispensable for understanding biological functions. Important physical techniques for research into the atomic-level analysis of biological structures center on X-ray crystallography, small-angle X-ray scattering (SAXS) and X-ray absorption fine structure (XAFS).

Proteins are a major component of biological macromolecules; they comprise 20 kinds of amino acids, many of which have been sequenced during the past few decades. However, greater knowledge about the three-dimensional structures of macromolecules is an essential step towards better understanding their functions. In this respect, the use of X-ray crystallography, to compile atomic-level information about three-dimensional macromolecular structures, is fundamental to advanced research in fields such as protein engineering and drug design. SAXS provides a complementary technique for analyzing the three-dimensional structures of macromolecules in solution at a molecular resolution, while XAFS reveals local structures around metal ions at an atomic level of resolution.

The Institute of Physical and Chemical Research (RIKEN) has designed and developed two SPring-8 beamlines for structural biology. BL45XU is designed for protein crystallography and SAXS, while the BL44B2 is concerned with time-resolved macromolecular crystallography and XAFS.

2 RIKEN Beamline I (BL45XU)

2.1 Beamline Design of RIKEN Beamline I

RIKEN beamline I (BL45XU) uses synchrotron radiation produced by two undulators, arranged in series, and has two experimental stations: one for protein crystallography and the other for SAXS. Both experimental stations can be used at the same time. The beam for BL45XU's protein crystallography station is provided from a “unique” trichromator that is optimized for experiments involving MAD (Multiple-wavelength Anomalous Diffraction).1,2 The design aims to minimize systematic errors that would prevent the successful application of MAD.

Figure 1 illustrates the beamline layout. The two undulators make it possible to supply different energy beams for MAD experiments. They can be operated independently to supply beams at different energies.3 Fundamental harmonics allow each undulator to emit beam energies in the range from 6 keV to 14 keV.

Dichromatic synchrotron radiation emitted from the two undulators is split by a transparent diamond monochromator.4,5 This beam-splitter acts as a first monochromator for the SAXS branch. The monochromatized undulator beam makes it possible to obtain high-flux data in an extended small-angle region. The structure of proteins in solution under various conditions is studied in this station. A CCD-detector with an X-ray Image Intensifier has been introduced to record diffraction and scattering patterns at the SAXS station. Moreover, RIKEN has collaborated with Professor S. Tanimori of the Tokyo Institute of Technology to develop a micro-strip gas chamber (MSGC),6 which is expected to provide a next-generation photon counting device.

The beam that passes through the thin-diamond crystal monochromator is guided to the trichromator beamline for protein crystallography.

2.2 Trichromatic Protein Crystallography at RIKEN Beamline I

Protein crystallography seeks to establish fundamental rules for the formation of three-dimensional structures. Compiling data about large numbers of three-dimensional structures underpins efforts to build a systematic classification of their properties and better understand associated biological functions. The main problems to be solved in protein crystallographic analysis include understanding the processes of crystallization, establishing the cryo-condition, developing hardware and software technology that enables

more effective and accurate data collection, phasing of diffraction spots, and tracing amino acid residues in electron density maps to fix their three-dimensional structure. By using the MAD with synchrotron radiation it is possible to phase diffractin spots analytically. This is an elegant technique. However, as anomalous scattering has minimal contribution to diffraction intensity, accurate data measurement is essential. The main feature of RIKEN's trichromatic design is its attempt to minimize systematic errors in MAD measurements that might arise from the setting of wavelengths and crystal orientation, change of crystallinity by time, and radiation damage.

MAD's trichromatic approach allows the sequential collection of three data sets from a protein crystal with fixed orientation. Two insertion devices are necessary for tuning, since two of the three wavelengths are close to the absorption edge of the crystal's metal ions, while other is significantly far from the edge. Three monochromatized radiation are brought out from dichromatic synchrotron radiation with trichromator, and the radiation are sequentially supplied through the beam choppers. The three data sets are collected in series to avoid interference amongst the wavelengths.

Successful MAD-experiments require specialized detectors. A fast read-out two-dimensional detector is presently the best choice for the high accuracy MAD measurements. RIKEN has developed and evaluated a multiple array CCD X-ray detector (MCCDX) to record protein crystal diffraction patterns.7

2.3. Present Status of RIKEN Beamline I

The installation of RIKEN Beamline I took place between August 1996 and June 1997. Commissioning of the beamline with synchrotron radiation began in July 1997. The initial test of the beam-splitter and trichromator were performed using diamond crystals with respective thicknesses of 0.7 mm and 1 mm. In July 1997, the diamond beam-splitter successfully splits the undulator beam to supply the SAXS and protein crystallography experimental stations. Moreover, the trichromator was able to supply three wavelengths to the protein crystallography experimental station. These three wavelengths were observed on a fluorescent screen and their energies confirmed by a solid state detector. Figure 2 shows the first diffraction image from a protein crystal. By the end of 1997, research had begun at both experimental stations.

3. RIKEN beamline II (BL44B2)

RIKEN beamline II (BL44B2) is dedicated to time-resolved macromolecular crystallography experiments using white radiation (Laue diffraction technique), and the XAFS of the dilute metalloproteins in solutions. It is also used for monochromatic data collection in routine macromolecular crystallography. Figure 3 provides a schematic plan of the beamline.

White X-rays from the B2 bending magnet are either focused with a 1m-long platinum-coated bent-cylindrical mirror (white X ray mode) or first monochromatized with a fix-exit double crystal monochromator (DCM) then focused by the same bent-cylindrical mirror. The DCM's first crystal can be moved in or out depending on experimental requirements. The mirror's glancing angle (2-5 mrad), radius of curvature (3000-7000 m), and vertical position (15mm) can be adjusted for the optimum focusing at the sample position.

Photon flux at the sample position (1 mm2) is estimated to be 1015 photons/sec (7-30 keV, white X-ray mode), and 1012 photons/sec in 0.1% b.w. (@20keV, monochromatic mode). The white X-ray mode is suitable for time-resolved Laue crystallography, with a typical exposure time using an image plate detector being less than 100 microseconds. XAFS experiments and routine macromolecular crystallography are performed with monochromatic X-rays, and energy resolution is in the order of 10-4. XAFS experiments in fluorescence or transmission modes are feasible in the energy range of 6-30keV.

The construction of RIKEN beamline II began at the end of 1996 and the radiation shield hatch was completed by March 1997. By the end of 1997, construction had been completed and the optical components, the inter-lock system and the control system were operational. Beamline commissioning with synchrotron radiation began in February 1998.

References

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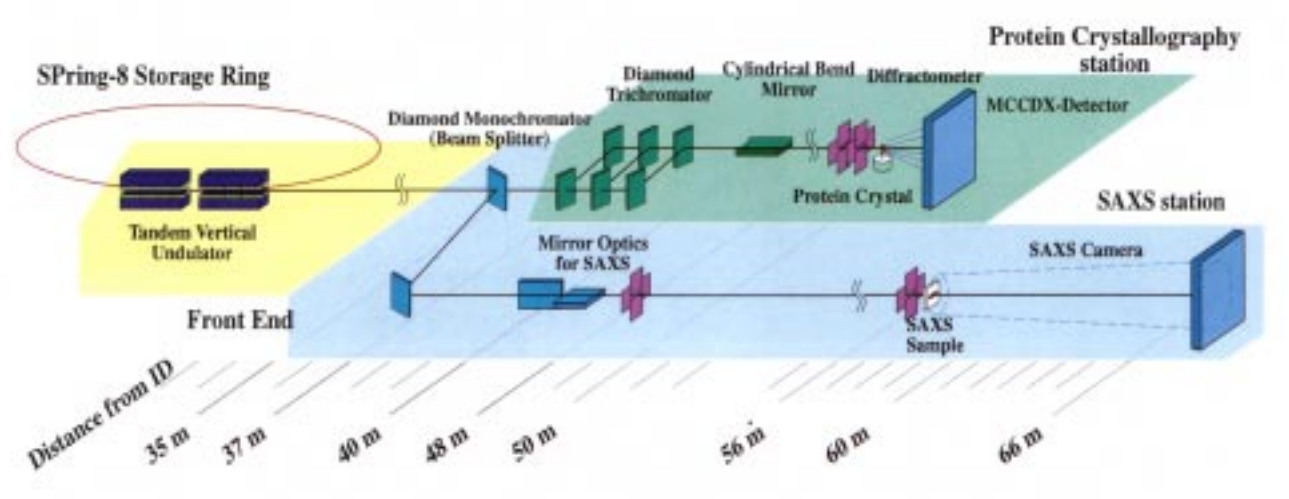

Fig.1. Bird's eye view of RIKEN beamline I (BL45XU).

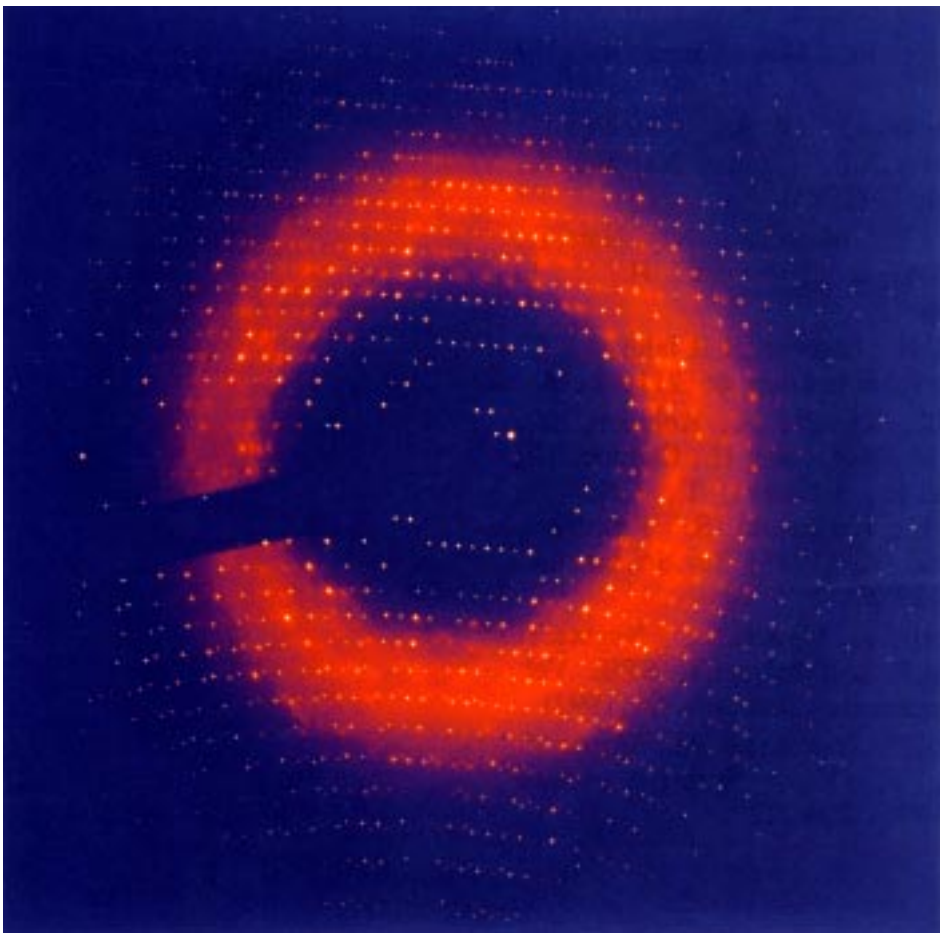

Fig.2. First diffraction image of the protein crystal at experimental station of BL45XU. Sample crystal is lysozyme of its tetragonal crystal form.

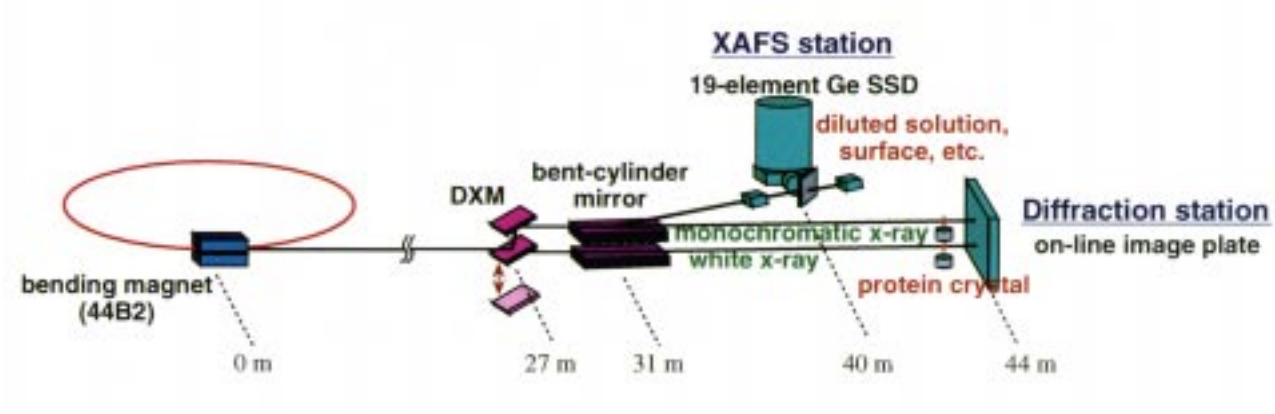

Fig.3. Bird's eye view of RIKEN beamline II (BL44B2).