RIKEN Structural Biology II (BL44B2)

1. Overview

The SPring-8 bending magnet beamline BL44B2, the RIKEN beamline II, is dedicated to white (Laue) and monochromatic macromolecular crystallography, and fluorescence and transmittance XAFS experiments [1]. This beamline has two experimental stations for diffraction and XAFS experiment modes, which are operated in time-sharing manner. In the diffraction mode, white X-rays from a bending magnet are focused in the diffraction hutch with a 1m-long platinum-coated bent-cylinder mirror (white X-ray mode, 6-25keV), or monochromatized with a fix-exit double crystal monochromator then focused with the same mirror (monochromatic X-ray mode, 6-20keV). In the XAFS mode, the monochromatized beam is reflected by the mirror at grazing angle between 0-5.5 mrad for optimum harmonic rejection. Here we summarize recent improvements to the beamline, which were carried out during 1999.

2. A CCD-Based Area Detector for Macromolecular Crystallography

In order to speed up diffraction data collection, we planned to adopt an electronically readable detector, and eventually installed a CCD-based area detector (marccd165) in June 1999. The full-frame readout time of the CCD detector (~3sec) is much faster than that of previous Imaging-Plate-based detector (~5min). Thus, data collection has been accelerated about 10 times (10-30 datasets per day with the CCD as against 1-3 datasets per day with the IP). This improvement led to tremendous increase of data output and to effective data collection with useful feedback from previous results especially in the case of the MAD applications (see Section 3). The specifications of the marccd165 are as follows.

Table 1. Detector Parameters of Marccd165

Type	Single taper, round aperture
Active Area (mm)	ϕ165
Number of Pixels	2048 × 2048
Pixel Size (mm)	0.079 × 0.079
Taper Ratio	2.9
CCD Type	Loral CCD485
Operating Temp.	-70ºC
Readout Time	3.0sec (2k×2k)

3. MAD Applications

Owing to the successful installation of the marccd detector, multiwavelength anormalous diffraction (MAD) experiments became much more effective than ever before. Because at least three datasets at different wavelengths are needed for the MAD applications, at best, users were only able to collect one MAD dataset for only one sample per day using the IP detector. At times the samples were damaged by long-time x-ray exposures even at cryo temperatures, which eventually causes the whole dataset to be unusable for further data analysis. The CCD detector accelerated data collection about 10 times, so that users are able to obtain a MAD dataset without serious damage to the sample. Furthermore, they can easily feed back their results to the next experiment even during a 24-hour beamtime; i.e. they can collect and process their datasets simultaneously, check the data quality, and can try other samples, wavelengths, crystal orientations, and additional images, etc. if they wish to. The quick feedback to the experimental conditions of MAD applications also accelerated the speed of structural determinations of macromolecular crystals (hopefully more than 10 times).

4. High-Resolution and Intermediate State Structural Studies

Other advantages of the beamline are,

1. high-resolution data collection capability at short wavelength (down to 0.6Å) X-rays, and
2. it provides equipment for trapping intermediate states in the macromolecular crystals (excitation light sources, a cryo-cooler, a microspectrophotometer, etc.).

Shorter wavelength X-rays are needed to perform high-resolution data collection because they can reduce systematic errors due to the absorption effect, and make data at higher values of $\sin\theta/\lambda$ accessible. We succeeded in collecting several datasets of protein crystals at atomic resolution (down to 0.9Å resolution) at the beamline. We are planning combined uses of short wavelength x-ray and a He cryostat for high-resolution data collection, which facilitates the deconvolution of thermal motion and bonding effects.

Several instruments are equipped for structural studies on intermediate states in protein crystals. Either monochromatic or white X-rays can be used when needed. The Nd YAG-laser-pumped OPO is used for excitation experiment in the UV-visible region. A freeze-trap experiment is feasible at 100K, and will be feasible at 40K in the near future.

References

[1] S. Adachi *et al.*, SPring-8 Annual Report (1996) 239.

Light Source:	Bending magnet
Optics:	Monochromator & Mirror
X-rays at Sample:	
Size:	0.1mm × 0.1mm
Energy:	6-20 keV

Facilities in Experimental Stations:

Detector:

diffraction experiments: marccd165, R-AXIS4

XAFS experiments: Ortec 19-element Ge-SSD

Crylstat, Nd YAG-laser-pumped OPO, dye laser, workstations, microspectrophotometer