Proc. Int. Symposium on Advances in Neutron Scattering Research, Tokai, 2000
J. Phys. Soc. Jpn. **70** (2001) Suppl. A pp. 459–461

Neutron Diffractometer for Biological Crystallography-3 (BIX-3)

Ichiro TANAKA, Kazuo KURIHARA, Toshiyuki CHATAKE and Nobuo NIIMURA

Advanced Science Research Center, Japan Atomic Energy Research Institute, Tokai, Ibaraki 319-1195, Japan

A neutron diffractometer for biological crystallography-3 (BIX-3) has been constructed at JRR-3M at Japan Atomic Energy Research Institute. It uses recent technical innovations, such as a neutron imaging plate and an elastically bent silicon monochromator developed by the authors. These have made it possible to collect diffraction data sets from crystals of Rubredoxin and Myoglobin in about one month measuring time. The data resolution of the data sets is 1.5 Å and allows the identification of hydrogen atoms with high accuracy. The data quality and required measuring time proves that BIX-3 is one of the best performance machines for neutron protein crystallography in the world today.

KEYWORDS: neutron diffractometer, biological crystallography, high resolution data, neutron imaging plate, elastically bent Si monochromator

§1. Introduction

The 3-dimensional structure of proteins and other biological macromolecules have been determined mainly by X-ray crystal structure analysis, which has extraordinarily contributed to life science. At the same time, these results clearly suggest that hydrogen atoms and the water molecules located around proteins and DNA play a very important role in many physiological functions. As it is very difficult to determine the positions of hydrogen atoms in protein molecules using X-rays alone, in most of the cases a detailed discussion of protonation and hydration sites can currently only be speculated upon. In contrast, it is well known that neutron diffraction provides an experimental method for directly locating hydrogen atoms.[1] Unfortunately, to date, there are relatively few examples of the use of neutron crystallography in biology, as a considerable amount of time is needed to collect a sufficient number of Bragg reflections.[2]

Several years ago one of us succeeded to collect diffraction data of HEW-Lysozyme[3] by using the quasi-Laue neutron diffractometer (LADI)[4] at the Institute Laue-Langevan (ILL), France. From the point of view that monochromatic diffraction has the advantage of owning a better signal to noise ratio compared to quasi-Laue, especially for biological macromolecular crystallography, a high performance monochromatic neutron diffractometer (BIX-3) has recently been completed, which may be one of the best machines for neutron protein crystallography in the world. In this paper we report the way how BIX-3 was constructed[5] and compare specifications and some experimental results of BIX-3 with those of LADI.[6]

§2. Technical Aims and Necessary Requirements

Because of the weakness of diffraction intensity protein crystallography is one of the most difficult experiments in the field of neutron crystallography. In order to overcome this difficulty three items have been proposed and developed; (1) to increase intensity at the sample position for 5 mm square cross section because the size of protein single crystals is at most several mm cube; (2) high performance neutron detector to cover large area with higher S/N ratio, higher spatial accuracy and better neutron detective efficiency; (3) to establish systematic approach to grow protein single crystals up to the size of several mm cube. Among of these the third item may be neglected because it is beyond the range of this paper. The other items will be referred in concrete in the following chapter of design.

Taking the above problems into consideration, we could reach a conclusion of necessary requirements for a practicable neutron diffractometer.

1) It should be possible to separate the nearest neighbor Bragg spots on the detector using the step-scanning method, to index them and to integrate

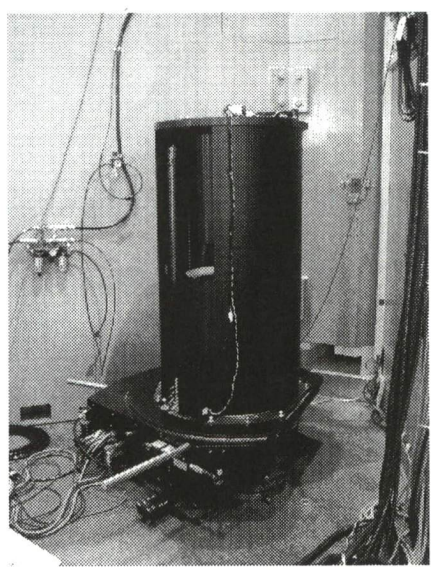

Fig. 1. BIX-3 photograph without detector shield.

intensity properly, which are diffracted from single crystals of 90 Å in the maximum unit cell parameter.
2) The measurement period should be approximately one month to collect data better than 2 Å resolution.

§3. Design[5)]

The most characteristic and elaborate design is the vertical arrangement of the main part of the diffractometer (Fig. 1), which made BIX-3 much more effective and powerful machine. The vertical design simultaneously solved the flux problem by making the sample position close to the monochromator, the shielding problem by a sufficiently minimum quasi-cylindrical detector shield and by a biological shield partially surrounding BIX-3, and the space sharing with the next machine, respectively.

The sample setting arrangement is also a noticeable. In order to read out the neutron imaging plate (NIP) in the lower part of the instruments automatically, the goniometer with the sample crystal is set upside down. For the simplicity around the machine the crystal alignment is performed at a remote alignment device equivalent to the real goniometer supporting frame.

Some of the distinct parts are explained as below.

3.1 Monochromator

In order to obtain more intensity of neutrons on biological macromolecule samples, the size of which is 5 mm cube at most, an elastically bent perfect silicon crystal using a piano wire tension has been developed and found to be very effective.[7)] It permits to focus on to the sample position. By bending two stacked silicon crystals we succeeded in increasing the neutron intensity by a factor of 1.6 compared to an optimally bent single silicon plate (Table I).[8)]

3.2 Detector -neutron imaging plate (NIP)-

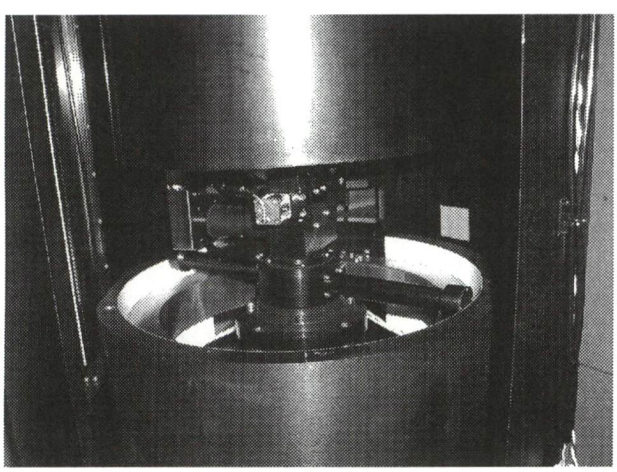

Fig. 2. The reading part of BIX-3.

The development and application of NIP's[9)] dramatically improved the detection of neutrons. For the diffractometer BIX-3 two sheets of NIPs are stacked on a cylindrical Al-base. The length of the NIP in the circumference direction is 1000 mm and the camera radius is 200 mm (Table I). Due to the high spatial resolution of the NIP the downsizing of a machine could be realized. One drawback of the NIP concept is the sensitivity to γ-rays, thus a shielding against them was necessary especially in the reactor hall.

For the reading procedure the NIP moves downwards along the cylindrical axis. The time required for the reading procedure is about 5 min (3 min for reading, 1 min for initialization and 1 min for NIP movement). Totally it takes about 5 min to reading precesses except exposure. In Fig. 2 an NIP and a rotating rod for a path of laser and photostimulated luminescence (PSL) from NIP is shown.

3.3 Shielding

After basic experiments at the planned site for BIX-3, shielding method was designed as follows. The instrument shield is composed of two layers, neutron shielding of 50 mm thick borated resin (B_4C + adhesive) outside and γ-ray shielding of 50 mm thick lead inside. The background from the environment in the reactor hall is relatively low, because BIX-3 is so small that it can be set near the biological shield made of heavy concrete.[5,6)]

Moreover in order to shield secondary γ-ray from the sample, a lead plate of 1 mm thick stuck on an Al base was set at a distance of 30 mm from the cylindrical neutron imaging plate (NIP). This thickness gives the best S/N ratio for the NIP.[10)]

§4. Experiments

As a typical measurement procedure, step scanning by 0.3 deg has been adopted. This procedure can be done by a full-automated system without any labor. From Rubredoxin (Rd)[11)] and Myoglobin (Mb) proteins we collected data sets of about 1000 frames at BIX-3, respectively. In both cases, data collection was done around two axes in order to collect sufficient independent reflections.

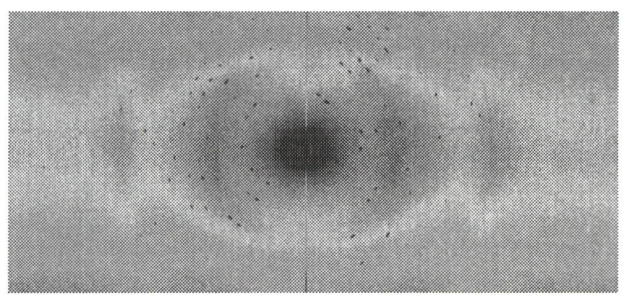

Fig. 3. A diffraction pattern of Rubredoxin crystal.

§5. Results and Discussion[6)]

It is not easy to compare the performances of the monochromatic and quasi-Laue method from considerably limited data. But by considering some basic quantities a comparison can be carried out.

5.1 Advantage from principle

For the quasi-Laue method realized in the diffractometer LADI the wavelength band is approximately from 3 to

TableI. Specification comparison between BIX-3 and LADI.

	BIX-3	LADI
incident neutron principle	monochromatic elastically bent silicon Si(111/311)(2.35/1.23 Å)	quasi-Laue multi-layer (Ti and Ni) (3-4 Å)
intensity [n/sec/cm^2]	3×10^6 ($\Delta\lambda/\lambda = 0.019$)	3×10^7 ($\Delta\lambda/\lambda = 0.28$ at 3.5 Å)
camera radius	200 mm	159 mm
detector	neutron imaging plate (NIP) 500 mm × 450 mm (2 plates)	neutron imaging plate (NIP) 200 mm × 400 mm (4 plates)
the max detector angle (2θ)	143 deg	144 deg
data resolution	1.2 Å/0.6 Å	1.6 Å
detector reading side	incident: front data read: front	incident: rear data read: front
S/N ratio	22	1 (as a unit)
data collecting period	25 days (d_{min}=1.5 Å)	10 days (d_{min}=2.0 Å)

TableII. Data quality comparison between BIX-3 and LADI by the use of factors of FOM in the last 3 lines.

	BIX-3	BIX-3	LADI
protein	Rd	Mb	Lysozyme
crystal volume V_C [mm^3]	4	6	6
measurement time T [day]	35	22	10
resolution limit d_{min} [Å^3]	1.5	1.5	2.0
wavelength band $\Delta\lambda$ [Å]	0.046	0.046	1.0
d_{min}^{-3}	2.4	2.4	1.0
$(\Delta\lambda)^{-1}$	22	22	1.0
$(T/V_C)^{-1}$	1.0	2.4	5.3

4 Å. On the one hand side the time required is short, on the other hand side the incoherent scattering background becomes high because of the wider $\Delta\lambda$. In addition ones run into the risk of overlapping Bragg reflections. In case of monochromatic method those overlaps never occurs because of step scan. The S/N ratio is better in the monochromatic case and integration can be calculated more precisely than in the Laue case. According to a simple calculation the S/N ratio of BIX-3 is about 20 times better than that of LADI because the wavelength band which contributes to Bragg reflection is about 3 % of the LADI case (Table I). Here N of S/N means background level.

5.2 Advantage from data

Figure 3 shows an example of data frames of a Rd crystal taken at BIX-3. The highest resolution of measurable Bragg reflections is about 1.5 Å. In Mb data set the resolution was almost the same.

To compare performances from raw data of different samples on different machines using different data reduction procedures while suppressing artifacts as much as possible, a figure of merit (FOM) can be introduced. The FOM is a function of the following values; resolution limit d_{min}, crystal volume V_C, measurement time T and wavelength width $\Delta\lambda$ (Table II).

$$FOM = f(d_{min}^{-3}, (\Delta\lambda)^{-1}, (T/V_C)^{-1}) \qquad (5.1)$$

Here, d_{min}^{-3} corresponds to N_r/V_a, the number of observed independent reflections Nr against the asymmetric cell volume Va. This ratio is roughly proportional to the number of parameters to be refined during structure refinement. $\Delta\lambda$ corresponds the used wavelength band which is proportional to background. As the third factor T/V_C measurement time per unit crystal volume,supposed that each crystal scan during the measurement was done in the same way. This FOM formula can be used to describe the overall data quality obtained from a sample taken at a neutron diffrcatometer for biology.

§6. Summary

Considering the FOM the total performance of BIX-3 diffractometer, due to the special design, is considerably better than the neutron diffractometer LADI at ILL in France, known as the highest intensity research reactor in the world. BIX-3 is installed at a relatively weak beam port (1G-A) at the middle class research reactor JRR-3M. The resulting instrument, nevertheless, permits the collection of high resolution data sets in a reasonable machine time because technical key points were effectively picked up, solved and developed one by one.

Acknowledgements

The authors would thank Prof. R. Bau, Dr. A. Ostermann and Prof. F. G. Parak for sample offers.

1) N. Niimura: J. Phys. Soc. Jpn. **70** (2001) Suppl. A. 396.
2) N. Niimura: Curr. Opin. Struct. Biol. **9** (1999) 602.
3) N. Niimura, Y. Minezaki *et al.*: Nature Struct. Biol. **4** (1997) 909.
4) D. A. A. Myles, M. S. Lehmann, C. Wilkinson *et al.*: Physica B **241-243** (1998) 1122.
5) I. Tanaka, N. Niimura *et al.*: J. Phys. Chem. Solids **60** (1999) 1623.
6) I.Tanaka, N. Niimura *et al.*: to be submitted.
7) N. Niimura, I. Tanaka, Y. Karasawa and N. Minakawa: Physica B **213-4** (1995) 929.
8) I. Tanaka, F. U. Ahmed and N. Niimura: Physica B **283** (2000) 295.
9) N. Niimura, J. Miyahara, K. Takahashi *et al.*: Nucl. Instr. Meth. A **349** (1994) 521.
10) Y. Haga, S. Kumazawa and N. Niimura: J. Appl. Cryst. **32** (1999) 878.
11) K. Kurihara, I. Tanaka, R. Bau, N. Niimura *et al.*: J. Phys. Soc. Jpn. **70** (2001) Suppl. A. 400.