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Neutron protein crystallography in JAERI

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Abstract. Neutron diffraction provides an experimental method of directly locating hydrogen atoms in proteins. After developing an original neutron detector (neutron imaging plate) and a novel practical neutron monochromator (elastically bent perfect Si monochromator), BIX-type diffractometers which were equipped with these tools were efficiently constructed at JRR-3 in Japan Atomic Energy Research Institute (JAERI), Japan and they have finished many protein crystallographic measurements and interesting results have come one after another. At the same time a method of growing large protein single crystals and a database of hydrogen and hydration have also been developed. In the near future, a pulsed neutron diffractometer for biological macromolecules has been proposed at J-PARC in JAERI.

Keywords. Crystallization macromolecules; crystallographic databases; crystal structure neutron diffraction and scattering methods; instruments for biophysics.

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1. Introduction

The three-dimensional structure determinations of biological macromolecules such as proteins and nucleic acids by X-ray crystallography has improved our understanding of the many mysteries involved in life processes. At the same time, these results have clearly suggested that hydrogen atoms and water molecules around proteins and nucleic acids play a very important role in many physiological functions. However, since it is very hard to determine the positions of hydrogen atoms in protein molecules using X-rays alone, a detailed discussion of protonation and hydration sites can only be speculated upon so far. In contrast, it is well-known that neutron diffraction provides an experimental method of directly locating hydrogen atoms, but unfortunately up to now there are relatively a few examples of neutron crystallography in biology since it takes a considerable amount of time to collect a sufficient number of Bragg reflections. The general subject of neutron protein crystallography has been reviewed by other authors [1]. These articles are helpful in understanding the historical background of this area of research.

In JAERI, the development of a new detector – neutron imaging plate (NIP) was a breakthrough [2,3], and the elastically bent perfect silicon (EBP-Si) monochromator contributed in obtaining the monochromatic neutron beams more effectively [4]. The general features of NIP and EBP-Si monochromator are reviewed. By utilizing NIP and EBP-Si monochromator organically, several high performance neutron diffractometers dedicated to biological macromolecules (BIX-2, BIX-3 and BIX-4) have been constructed at JRR-3 reactor in JAERI [5,6] and these have enabled high resolution (from 1.5 Å to 2.0 Å) structural analyses of several proteins. The crystal structures of myoglobin, wild type of rubredoxin and mutant of rubredoxin, human lysozyme, cubic insulin, DsrD and so on have been carried out using BIXes. From these studies, very interesting topics relevant to hydrogen and hydration in proteins have been extracted from the structural results [2,7–15]. In addition, a method of growing large and good single protein crystals [12–14,16] and a development of a database of hydrogen and hydration using neutron protein crystallograpic data is introduced. Finally, a diffractometer for biological macromolecules with higher efficiency has been proposed at the next generation neutron source J-PARC, which is under construction in JAERI [17].

2. Instrumentation for the BIX-type diffractometers

The recent development of NIP was a breakthrough in neutron protein crystallography (table 1). NIP was produced only by mixing neutron converter Gd into X-ray imaging plate (IP) skillfully, and optimized for neutron protein crystallography [2,3]. It can be read by reading machines for X-ray IPs.

The development of EBP-Si monochromator was also important (table 2, figure 1) [2,4]. An EBP-Si monochromator is excellent because of the better transmission of thermal neutrons, which enables one to share the whole beam cross-section into a one beam port [6].

The first application of the NIP was a structure determination of tetragonal hen-egg-white lysozyme using a quasi-Laue diffractometer, LADI, at Institut Laue-Langevin (ILL) in Grenoble [18]. However, we found that the background level is inherently high in a Laue-type diffractometer, especially for protein crystallography,

Table 1. Neutron imaging plate (NIP) specification table.

Detector type	Integration
Neutron converter	Natural gadolinium (Gd)
Neutron capture efficiency ($\lambda = 1$ Å)	$\sim 80\%$
Positional resolution	Less than 0.2 mm
Linearity	Less than 0.1% (all areas)
Counting loss limit	More than 10^{10} cps/cm^2
Effective area	No limitation (in principle)
γ-ray sensitivity	1/20 of neutron (for γ-rays of energies higher than 300 keV)
Additional instrumentation required	Laser reader
Structure of detector	Flexible (film-based)

Table 2. A comparison table between EBP-Si and PG.

	Elastically bent perfect silicon (EBP-Si)	Highly oriented pyrolytic graphite (HOPG)
Mosaicity	Controllable	Fixed
Reflected intensity	Adjustable to the same extent as HOPG (plate-stacking increases reflectivity)	Large
Focal length	Controllable	Divergent
Reflection plane	(1 1 1), (3 1 1) and others	(0 0 2) only; contamination of $\lambda/2$ reflection
Pseudo-focusing in the vertical direction	Possible	Possible
Absorption	Negligible (even thick plate)	Not negligible (affects the down stream)
Price	Reasonable (about 1/10 of HOPG)	Expensive

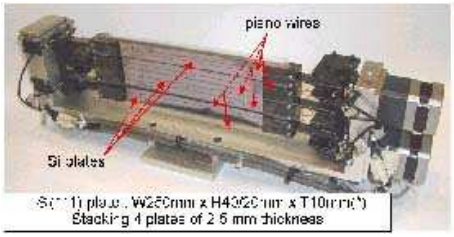

Figure 1. A photo of EBP-Si monochromator and its bender.

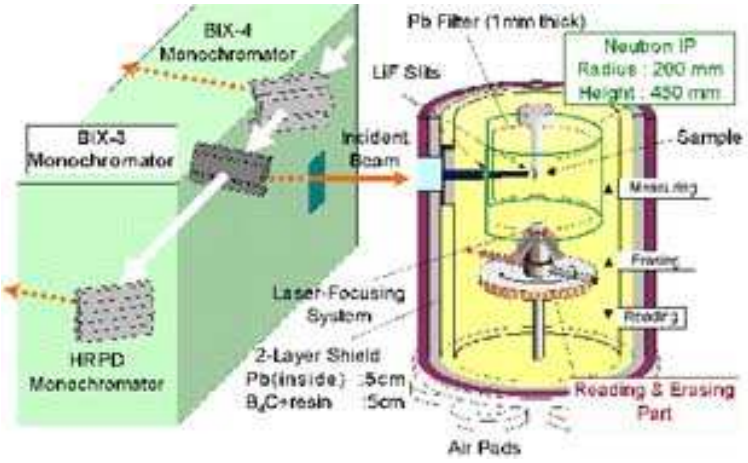

Figure 2. Schematic view of BIX-3.

because of the high level of hydrogen in the samples studied. In order to overcome this problem, we have constructed high-resolution neutron diffractometers dedicated to biological macromolecules (BIX-type diffractometers), which equip NIP and EBP-Si for monochromatized neutron beam, at JAERI [2,5,6]. In figure 2, a schematic view of BIX-3 is shown. The samples measured by BIX-type diffractometers since 1996 are listed in table 3. The performance of BIX-type diffractometers was excellent not only for proteins but also for organic compounds and a small amount of powder sample as hydrous silicate (less than several mg) [2,7–15,19].

3. Results from the BIX-type diffractometers

The neutron diffraction data taken by BIX-3 and BIX-4 that were measured for a period of 1–2 months were very good because their best resolutions are around 1.5 Å from normal proteins (approx. 6–35 kDa) with less than 6 mm^3 volume crystals (see table 3). Some of the Fourier maps of rubredoxin and met-myoglobin are shown in figure 3. The difference between hydrogens and deuterium [8], and the patterns of hydrations including their orientations [9,11,13] are clearly seen.

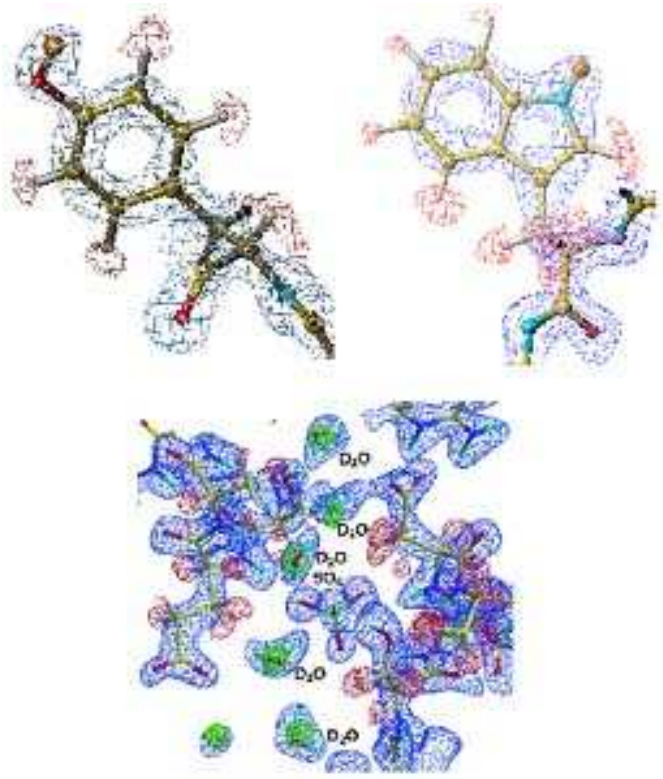

Figure 3. Hydrogens (red contours), deuteriums in Tyr10 (top left), Trp3 (top right) of rubredoxin (WT) and waters in met-myoglobin (bottom). Green contours overlapped are X-rays.

Table 3. Sample list of BIX-type diffractometer in JAERI.

Sample	Unit cell/ space group (Å)	$d_{\min}$ (Å)	Crystal size (mm^2)	Data collection (days)	Machine	Status (as of Nov. 2003)
HEW lysozyme pH 4.9	$79 \times 79 \times 38/P4_32_12_1$	2.0	6	140	BIX-2	Maeda *et al* (2001)
HEW lysozyme pH 3.4	$79 \times 79 \times 38/P4_32_12_1$	2.0	6	140	BIX-2	Under refinement
HEW lysozyme pH 7.0	$79 \times 79 \times 38/P4_32_12_1$	2.0	6	140	BIX-2	Under refinement
HEWL-substance analog complex	$79 \times 79 \times 38/P4_32_12_1$	2.0	6	140	BIX-2	Under refinement
Rubredoxin (wild type)	$34 \times 35 \times 44/P2_12_12_1$	1.5	4.4	35	BIX-3	Kurihara *et al* (2001)
Met-myoglobin	$65 \times 31 \times 35$ $\beta = 106^\circ/P2_1$	1.5	6.3	22	BIX-3	Ostermann *et al* (2002)
Rubredoxin (mutant)	$35 \times 36 \times 43/P2_12_12_1$	1.6	6.0	31	BIX-3	Chatake *et al* (2002)
Human lysozyme	$34 \times 57 \times 61/P2_12_12_1$	1.8	2.1	17	BIX-3	Chiba *et al* (submitting)
Insulin (pig)	$79 \times 79 \times 79/I2_13$	1.6	18	13	BIX-3	Maeda *et al* (2004)
Cytochrome C'	$53 \times 53 \times 182/P6_522$	2.8	2.9	20	BIX-3	Under processing
DsrD	$61 \times 65 \times 47/P2_12_12_1$	2.4	1.7	70	BIX-3	Chatake *et al* (2003)
DNA d(CCATTAATGG)	$33 \times 33 \times 96/P3_221$	3.0	2.8	32	BIX-4	Under refinement
Endopolygalacturonase I	$46 \times 52 \times 37$ $73^\circ, 69^\circ, 69^\circ/P1$	1.5	4.5	37	BIX-4	Under refinement
Trypsin inhibitor (bitter gourd)	$23 \times 24 \times 28$ $93^\circ, 100^\circ, 101^\circ/P1$	1.8	0.3	22	BIX-4	Under processing

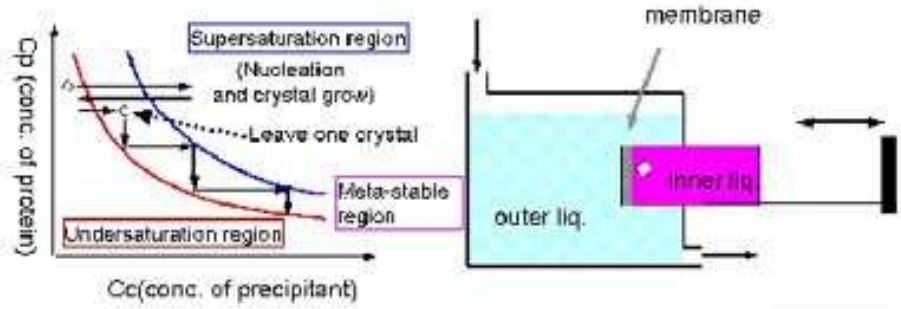

Figure 4. A principle of growing a large and good protein single crystal in the phase diagram (left), and a schematic view of a size-free protein crystallization system (right).

4. Other important developments around neutron protein crystallography

The main problem with neutron protein crystallography is to obatin a large single crystal as a sample. To overcome this, a size-free protein crystallization system has been under development. In figure 4, a principle of growing a large and good protein single crystal in the phase diagram [2,12–14,16] and a schematic view of the system are shown.

When one obtains much hydrogen positional data through protein neutron crystallography, it is very difficut to deal with them correctly, and the present database system like PDB is not sufficient to study hydrogen-related studies such as hydrogen bonding. After inputing the author's neutron protein crystallographic data [8–10], hydrogen and hydration database (HHDB) have been developed. In figure 5, a window of hydrogen distribution of met-myoglobin is presented with an example of bifurcated hydrogen bond, which clearly shows strong and weak hydrogen bonds in their angles and distances [20].

5. Near future plan in J-PARC – the next generartion neutron source

A diffractometer for biological macromolecules and organic compounds with unit cell dimensions less than 135 Å (BIX-P1), which is expected to gain an efficiency of around 100 times larger than neutron biological diffractometers BIX-3 and BIX-4 installed at JRR-3 in JAERI, has been proposed for Japan Proton Accelerator Research Complex (J-PARC) with the power of 1 MW in JAERI. The conceptional design of BIX-P1 has been completed [17].

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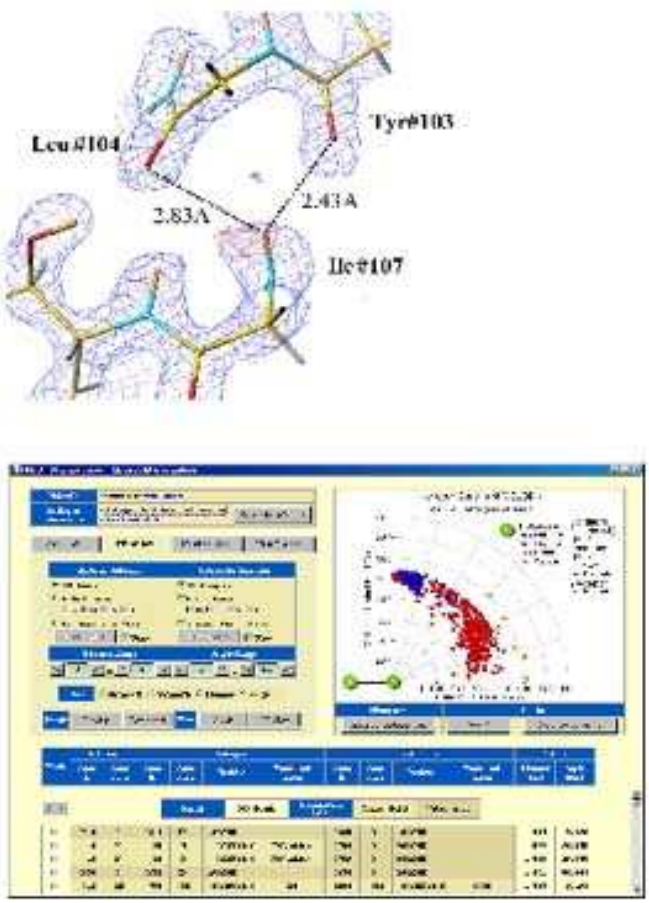

Figure 5. An example of bifurcated hydrogen bond in met-myoglobin (top) and a window of the hydrogen-bond distribution using hydrogen and hydration database (HHDB) (bottom).

ogy, Japan. The development of protein crystallization system was supported by R&D Fund for Regional Emerging Consortiums, Japan. The hydrogen hydration database (HHDB) was developed by the joint research of Japan Atomic Energy Research Institute (JAERI) and Japan Science and Technology Agency (JST) as part of the database development program of JST.

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