Construction of neutron diffractometer for biomacromolecules and its application to lysozyme crystal

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The neutron crystallography of biomacromolecules is expected to clarify the nature of hydrogen bonds, their stabilization by bound water molecules and so on because it can determine the positions of hydrogen atoms by referring X-ray diffraction result. But the experimental examples were very few because of the lack of incident neutron intensity. However, also in Japan, a demand of such kind of neutron diffractometer has been increased year by year. Recently more powerful reactor (JRR-3M) has started to be operated, that has enabled one to construct a following diffractometer, and to take neutron data set of enzyme protein hen-egg-white lysozyme by using it.

A dedicated diffractometer for neutron crystallography in biology (BIX) has been constructed at JRR-3M in Japan Atomic Energy Research Institute (JAERI). In this abstract elastically bent Si monochromator system should be explained especially. One of the favorable characteristics is that the reflectivity and the focusing point are varied by bending monochromator crystal elastically (Fig. 1& 2). Currently, the curvature R of the monochromator is set so that beam intensity at the sample position becomes maximum. Conventional monochromator PG (pyrolytic graphite) would not work so well in this case mainly because the angular divergence of the monochromatized beam is large. Overall specification of the diffractometer is shown in Table 1.

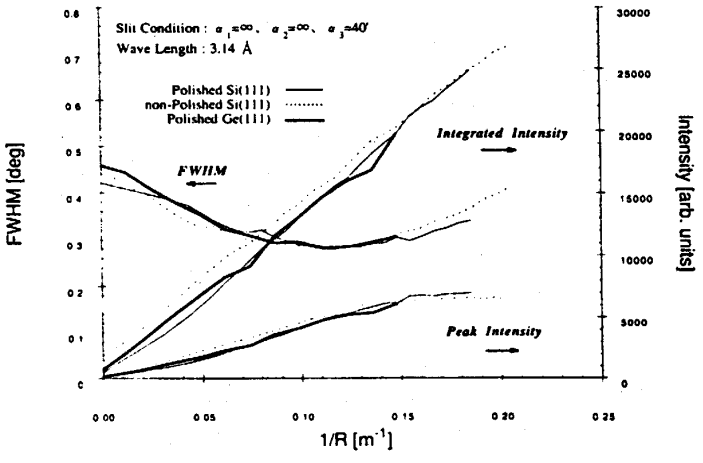

Fig. 1 : Bent perfect crystal rocking curve data on another diffractometer.

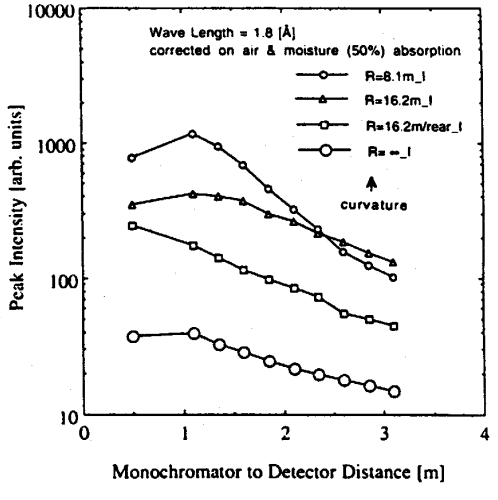

Fig. 2 : Focusing test of bent Si (111).

Monochromator	horizontally bent Si (111)	horizontally bent Si (220)
Wave length	λ : 1.73 Å	λ : 1.06 Å
Beam collimation	H40×W40 mm^2 at Monochromator, H5×W5 mm^2 at Sample	
Each distance	Monochromator to Sample=3100mm, Sample to Detector=600 mm	
Beam characteristics	angle divergence : 0.6° , $\Delta\lambda/\lambda = 0.037$ at λ =1.06 Å	
Intensity at sample position	5.9×10^6 n/cm^2/sec	1.4×10^6 n/cm^2/sec
Detector	^{3}He-gas-proportional : 2 sets active area : 250×250 mm^2/set, spacial resolution : 2 mm	
Detector shielding	material : 70mm(B_4C)+50mm(H), background : 0.1n/cm^2/sec	
Range of d-spacing	$d_{min}\geqq$1.5 Å	$d_{min}\geqq$0.9 Å
Maximum unit cell constant	150 Å	90 Å

Table 1 : Specification of newly constructed neutron diffractometer for biomacromolecules (BIX)

Hen-egg-white lysozyme was crystallized (tetragonal form : a=b=79.1 Å ,c=37.9 Å, Z=8) in D_2O buffer, the size of which is about 30 mm^3 in volume. Collection of Bragg reflections was carried out by step scan method in order to escape overlapping of spots and to improve signal/noise ratio. The step angle was 0.2 degrees and the exposure time was 20 to 30 minutes/step. Finally about 12,000 reflections of more than 2.1 Å d-spacing were collected for about 50 days.

Data reduction procedures such as peak search, indexing, integration of reflection intensity and so on were carried out so that calibrated structure factors could be obtained. As the examples of assessment of data, one part of low resolution data is shown in Fig. 3 & 4. The results suggest that enough reflections to determine the positions of hydrogen atoms were collected .

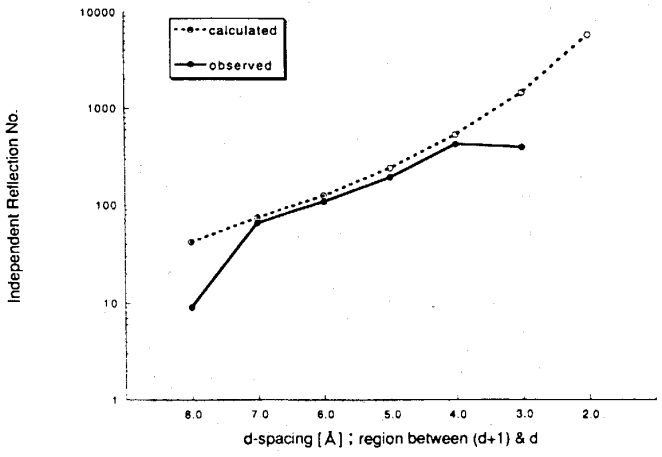

Fig. 3 : Comparison of numbers of independent reflections between observed and calculated ones.

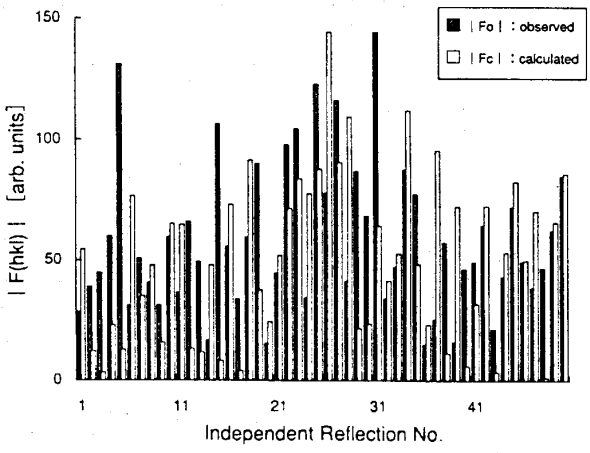

Fig. 4 : Comparison of structure factors between observed and calculated (model without hydrogens) ones. Errors are at most within several percents.