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Time-Resolved Protein Crystallography with Large-Angle Oscillations: an Application of a Protein Data-Collection System Using the Weissenberg Technique and a Large-Format Imaging Plate

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A diffraction-intensity data-collection system with synchrotron radiation X-rays utilizing the screenless Weissenberg technique and incorporating a large-format imaging plate is one of the most suitable apparatus for time-resolved protein crystallography with larger angle oscillations than hitherto described. The time resolution and data quality of the system have been tested using a tetragonal lysozyme crystal as a test sample in a flow-cell experiment at the bending-magnet beamline 18B at the Photon Factory, and a time resolution of 15 min is confirmed.

Keywords: large-angle oscillations; Weissenberg technique; imaging plates; short-wavelength X-rays; Photon Factory; tetragonal lysozyme; time-resolved studies.

1. Introduction

For time-resolved protein crystallography, data-collection systems with synchrotron Laue diffraction geometry have recently been developed (Helliwell *et al.*, 1989; Bourgeois *et al.*, 1996, and references therein). The Laue method is very powerful for studying fast reactions with a time scale of the order of picoseconds to milliseconds (depending on the scattering efficiency of the crystal sample). However, diffraction data sets collected using the Laue method are inferior in accuracy and completeness to those obtained with monochromatic radiation.

Unlike the Laue method, the large-angle oscillation technique, LOT (Weisgerber & Helliwell, 1993), with a monochromatic X-ray beam provides diffraction data with high accuracy and high completeness (Bradbrook *et al.*, 1995), and is applicable to time-resolved protein crystallography with a time scale longer than minutes or hours. This time scale is comparable with that of soaking processes of chemical reagents into protein crystals. These soaking processes may often increase the sample crystal mosaicity, which will affect the data quality in the Laue method. Application of the LOT is not seriously limited by the sample crystal perfection. A chemical reagent soaking process can be observed by X-ray crystal structure analysis with a flow cell (Hajdu *et al.*, 1987).

Among techniques using monochromatic X-rays, the screenless Weissenberg method (Sakabe, 1983, 1991) gives less-overlapping diffraction patterns of reflections in the large oscillation angle up to high resolution than other oscillation techniques. This is because the Weissenberg camera translates the detector synchronously with the crystal oscillation to avoid overlapping of recorded reflections. Therefore, the Weissenberg method is one of the most preferred techniques for time-resolved protein crystallography using the LOT for slow reactions (Hajdu & Andersson, 1993).

In order to realize the large oscillation range, the screenless Weissenberg camera designed for the LOT must have a large cassette radius because intervals of layer-lines should be wide enough to record many reflections separately. However, a large cassette inevitably requires a detector with a large active area. These requirements can be met by the diffraction intensity data-collection system originally developed for the time-resolved synchrotron Laue experiments by the present authors (Sakabe *et al.*, 1995). This system consists of a Laue camera with two cassettes of very large radii (430 and 1290 mm), large-format flat imaging plates (800 × 400 mm^2) and corresponding image readers. The system is installed in the bending-magnet beamline 18B at the Photon Factory, and can also be used for Weissenberg data collection with a monochromatic X-ray beam when the high-speed rotary shutter is held open (Watanabe, Nakagawa, Adachi & Sakabe, 1995). Here

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we describe preliminary evaluation of this data-collection system as an apparatus for recording as large as possible rotation angles in a single exposure.

2. Experimental

In order to evaluate our data-collection system we selected a hen egg-white lysozyme (HEWL) tetragonal crystal as a test sample. The crystal has space group $P4_32_12$ and the unit-cell dimensions are a = 79.1 and c = 38.0 Å (Cheetham, Artymiuk & Phillips, 1992). Experimental conditions are summarized in Table 1.

All data collections were carried out using short-wavelength X-rays (0.7 Å) in order to minimize X-ray absorption effects. We utilized the large cassette of the Laue camera to realize the large oscillation range required for the LOT. The large camera length with helium pass was also effective for dispersing the X-ray background noise scattered from the sample crystal and the reaction medium surrounding it. For each X-ray exposure, one large-format imaging plate was placed asymmetrically with respect to the primary X-ray beam in order to record a wider reciprocal space. In all the experiments one data set was collected using successive X-ray exposures on two imaging-plate sheets, on each of which the recorded oscillation range was 31° with an overlap of 2°. The total oscillation angle was 60° for one data set, which exceeded the minimum oscillation range of 45° necessary to obtain a unique data set for an HEWL tetragonal crystal aligned along the c axis. The coupling constant was 1.3° mm^{-1}. The imaging-plate sheet was fixed inside the cassette by evacuation from the back of the cassette.

In order to examine the absorption effect from the materials around the sample crystals, we collected reference data using a 0.7 × 0.4 × 0.8 mm crystal mounted in a shielded glass capillary with a very small amount of mother liquor. The oscillation number and speed of the Weissenberg photography were selected as 10 and 2° s^{-1}, respectively, and the exposure time for one imaging-plate sheet was 310 s. It took ~2 min to exchange the imaging-plate sheets, and thus the second exposure was started 8 min after the beginning of the first exposure. The shortest time resolution achieved under the experimental conditions mentioned above was 15 min.

The flow-cell experiment was designed in order to trace soaking processes of substrates into HEWL crystals in a reaction medium such as chitotriose solution. The sample crystal (0.7 × 0.7 × 1.2 mm) with its mother liquor was wedged on the slightly tapered inner surface of a 1.0 mm-diameter glass capillary without using fixing material such as Sephadex gel. The glass capillary was connected to a steel pipe on a special holder. The holder was designed so that it could be mounted on a goniometer head, and was similar to that made by Dr J. Hajdu of Oxford University. The mother liquor around the crystal was exchanged for the chitotriose solution at a flow rate of 0.3 ml h^{-1}, and the solution which flowed past the sample crystal emerged from the open end of the glass capillary (Edwards, 1993). Five data sets were obtained at 29 min intervals within 2.5 h. The solution exchange was started 11 min before the start of collection of the second data set. Each data set was collected under the same conditions, except for the oscillation number and the speed. An oscillation number of 10 and a speed of 2° s^{-1}, as for the reference data collection, were chosen for data set 1, while an oscillation number of 2 and a speed of 0.4° s^{-1} were used for data sets 2–5 to avoid sample crystal slippage.

Table 1
Experimental conditions.

X-ray wavelength	0.7 Å (Photon Factory, BL18B)
Cylindrical cassette radius	1290 mm
Effective imaging-plate area	400 (H) × 800 (V) mm (one large-format imaging plate)
Oscillation range	31° exposure $^{-1}$
Oscillation speed	2° s^{-1}
Oscillation number	10
Coupling constant for Weissenberg photography	1.3° mm^{-1}
Exposure time	310 s exposure $^{-1}$
Total oscillation angle	60° (with two imaging-plate sheets; 2° overlap)
Total data-collection time	15 min (including the time for imaging-plate exchange)

3. Results and discussion

Table 2 summarizes the data statistics of the flow-cell experiment together with the reference data after reduction and scaling with the *WEIS* program (Higashi, 1989). As described above, the oscillation range for one imaging-plate sheet is 31°, surprisingly large in spite of using short-wavelength X-rays (0.7 Å). This can be compared with the 90° range for a single exposure of concanavalin A by Weisgerber & Helliwell (1993) and the discussion of a full revolution being investigated by Bradbrook *et al.* (1995) using lysozyme as a test case. Table 2 shows that only two exposures for one data collection within 15 min are enough to record the reflections with 84% completeness up to 2.0 Å resolution and 71% up to 1.75 Å resolution. The R values between the flow-cell data and the reference data, 0.061–0.071, indicate that sufficiently high-quality data for structure refinements can be obtained using this data-collection system. The absorption effect from the solvent around the crystal in the flow cell can be neglected when short-wavelength X-rays (0.7 Å) are used. According to Cheetham, Artymiuk & Phillips (1992), the high-quality and high-resolution data at 1.75 Å are preferable for detecting real structure changes of HEWL caused by binding of chitotriose. Thus, this data-collection system is extremely suitable for time-resolved protein crystallography at high resolution for slow reactions.

During the flow-cell experiment, as mentioned above, we changed the oscillation number and speed. If the primary X-ray beam intensity fluctuates on a time scale of the order of seconds, the quality of the data sets depends on the oscillation number because of merging effects. Our experimental results indicate that the oscillation number has no effect on

Table 2
Data-collection summary.

Data set	Flow-cell experiment 1	2	3	4	5	Reference data
Substrate soaking	No	Yes	Yes	Yes	Yes	No
Soaking time (h)	–	0.2	0.7	1.2	1.6	–
Oscillation speed (° s^{-1})	2	0.4	0.4	0.4	0.4	2
Oscillation number	10	2	2	2	2	10
Observed reflections						
(20–2.0 Å)	13666	13565	13593	13539	13501	13681
(20–1.75 Å)	15888	15748	15762	15693	15665	15947
Unique reflections						
(20–2.0 Å)	7227	7192	7203	7181	7161	7308
(20–1.75 Å)	9060	9028	8995	8958	8963	9152
Completeness (%)						
(20–2.0 Å)	84.9	84.5	84.6	84.3	83.9	85.8
(20–1.75 Å)	72.1	71.9	71.6	71.3	71.3	72.8
R_{ext} value*	0.066	0.061	0.063	0.066	0.071	–
B-value difference	0.1	0.3	0.8	0.9	1.0	–
R_{int} value†	–	0.057	0.059	0.061	0.062	–

* $R_{ext} = \sum |F_{ref}(h) - F_x(h)| / \sum F_{ref}(h)$; F_{ref} = structure-factor amplitude of reference; F_x = structure-factor amplitude of flow-cell experiment, x = 1–5.
† $R_{int} = \sum |F_1(h) - F_x(h)| / \sum F_1(h)$; F_1 = structure-factor amplitude of flow-cell experiment 1; F_x = structure-factor amplitude of flow-cell experiment, x = 2–5.

the data quality. This may be due to the excellent stability of the Photon Factory ring, and is advantageous because the X-ray intensity of the primary beam can be increased, thus allowing a decrease in oscillation number. When the data-collection system described here is installed in a high-flux multipole wiggler beamline at the Photon Factory, the collection of one high-quality data set with a high resolution will take 3–4 min, including the time required for imaging-plate exchange, using sample crystals with similar cell dimensions and symmetry to those of HEWL tetragonal crystals.

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