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Protein crystallography using a multilayer monochromator

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A multilayer monochromator was installed on a bending-magnet beamline at the Cornell High Energy Synchrotron Source (CHESS) and was used to provide an unfocused pseudo-monochromatic X-ray beam for protein crystallography experiments. Datasets were collected from lysozyme at room temperature and human methylthioadenosine phosphorylase at 100 K. The wide energy bandpass of the multilayer allowed short exposure times, typically only a few times longer than on a focused multipole wiggler beamline. The diffraction images were processed using unmodified monochromatic data-processing software to yield datasets of good quality. These first measurements demonstrate that multilayer monochromators can be readily applied to the rapid structure determination of many typical-sized macromolecules.

Keywords: X-ray optics; multilayers; monochromators; macromolecular crystallography; protein crystallography.

1. Introduction

There is a high demand for beam time on macromolecular crystallography stations at synchrotron radiation sources. At CHESS the stations that support this field, as part of the MacCHESS facility, are heavily oversubscribed. Efforts are being made to develop systems that allow users to achieve their experimental goals more rapidly. The use of CCD detectors for macromolecular crystallography has been pioneered at CHESS (Walter *et al.*, 1995; Thiel *et al.*, 1995, 1996). They offer efficient detection of X-rays, coupled with a very short readout time. Recently, user-friendly graphical interfaces (GUIs) have been developed to allow users to quickly and intuitively run experiments (Szebenyi, Deacon *et al.*, 1997; Szebenyi, Arvai *et al.*, 1997) using these state-of-the-art detectors. For many projects the exposure time is now the rate-limiting step in the data-collection process.

Macromolecular X-ray diffraction data are generally collected in one of two modes: using either a stationary crystal with a polychromatic X-ray beam (the Laue method) (Helliwell, 1992), or a rotating crystal with a monochromatic X-ray beam (Arndt & Wonacott, 1977). The former is used primarily for time-resolved experiments (Ren & Moffat, 1994), while the latter is the standard for most other types of measurement. The oscillation method (Rossmann & Erickson, 1983) is the most commonly adopted experimental geometry, incorporating a single rotation-axis (the spindle) and an area detector. A highly monochromatic X-ray beam, provided by a silicon or germanium monochromator, is normally used. Multilayer technology can be used to provide a wider band-pass X-ray beam, although this has not previously been applied to protein crystallography. In this paper the possibility of reducing exposure times by using a wide-band-pass monochromatic X-ray beam from a multilayer in standard oscillation geometry is investigated and suitable macromolecular crystallography experiments are identified.

Table 1
Data-collection information for HEW lysozyme and human MTAP.

	HEW lysosome	Human MTAP
Space group	$P432_12$	$P321$
Cell dimensions	a = 79.0, b = 79.0, c = 38.0 Å	a = 122.5, b = 122.5, c = 45.2 Å
Mosaicity used	0.5°	0.75°
Crystal to detector distance	72.0 mm	117.0 mm
Exposure time	20 s	15 s
Oscillation angle	1°	1°
Redundancy	1.7	3.1
Number of images	23	162

2. Experimental

2.1. *Data collection*

A multilayer (No. XRO-673-6) was purchased from ECD (now Osmic Inc.). It consisted of 200 layer-pairs of tungsten and silicon, with a d-spacing of 24 Å, deposited on an optically polished electro-less nickel–aluminium substrate. It was installed on the bending-magnet station D1 at CHESS and was operated with no focusing, in a single-bounce geometry at a glancing angle of 1.2°. At one time the reflectivity of the multilayer was measured to be 50% at 8 keV. The energy band-pass of the multilayer was measured to be ~240 eV at 13.89 keV ($\Delta E/E$ = 1.7%), by scanning a silicon crystal. No significant Fresnel structure was observed. The higher-order harmonics were expected to be very small, since the multilayer was manufactured in 1983 and the substrate had a 10 Å r.m.s. roughness. The X-ray intensity was 7×10^{10} photons s^{-1} at 100 mA through a 0.3 mm collimator. A standard single-rotation-axis oscillation camera and the Princeton 1K CCD detector (Tate *et al.*, 1994) were used to collect X-ray diffraction data from protein crystals.

A dataset was collected at room temperature from a single-crystal of tetragonal hen egg-white (HEW) lysozyme mounted in a glass capillary (Table 1). Radiation damage soon became apparent and the data collection was stopped after 23 exposures. The images consisted of radially streaked diffraction spots (Fig. 1*a*), due to a convolution of the crystal mosaicity, the beam divergence and the $\delta\lambda$ (wavelength spread) contribution in Braggs law ($\lambda = 2d\sin\theta$). The $\delta\lambda$ contribution to this streaking can be calculated from $\delta\lambda = \lambda\cot\theta\,\delta\theta$. It may be possible to reduce the streaking by collecting stills in a narrow-band-pass Laue geometry, although this was not attempted. Nevertheless, the diffraction peaks appeared to be clearly resolved. A dataset was then collected from a single crystal of human methylthioadenosine phosphorylase (MTAP). The crystal was frozen in a fibre loop at 100 K (see Rodgers, 1994, for a review of cryocrystallography techniques). The detector was moved back and offset with respect to the direct beam to allow for the larger cell dimensions and yet still collect data to 2.6 Å resolution (Table 1). Again the

Table 2
Data-processing statistics for HEW lysozyme and human MTAP.

Tetragonal HEW lysosome				Human MTAP			
Resolution range	Number of reflections	$I/\sigma(I)$	R_{sym}	Resolution range	Number of reflections	$I/\sigma(I)$	R_{sym}
10.0–5.15	286	18.2	0.043	10.0–5.33	1256	27.2	0.077
5.15–4.19	293	15.5	0.058	5.33–4.35	1220	25.4	0.078
4.19–3.69	328	13.2	0.067	4.35–3.83	1181	19.5	0.082
3.69–3.37	326	11.5	0.079	3.83–3.50	1083	17.4	0.081
3.37–3.13	300	9.8	0.089	3.50–3.26	983	13.9	0.085
3.13–2.95	277	7.9	0.116	3.26–3.07	885	13.1	0.088
2.95–2.81	252	6.8	0.128	3.07–2.92	820	10.4	0.091
2.81–2.69	242	6.3	0.132	2.92–2.80	730	9.1	0.096
2.69–2.59	230	5.6	0.169	2.80–2.69	529	7.9	0.098
2.59–2.50	201	4.7	0.202	2.69–2.60	298	6.8	0.106
All *HKL*	2735	11.3	0.074	All *HKL*	8985	17.8	0.080

images consisted of clearly resolved diffraction maxima (Fig. 1*b*), despite the increased mosaicity usually associated with frozen crystals and the significantly larger cell dimensions of this particular protein. The spot size is increased here because of the larger mosaicity.

2.2. *Data processing*

The diffraction images were processed using the *HKL* program suite (Otwinowski & Minor, 1997). There was no ideal way of modelling the radially streaked spot profiles, nor of predicting the extra observed diffraction spots due to the energy bandwidth. A circular peak-profile region was defined with as large an area as possible, while keeping the number of predicted overlaps to a minimum. An increased mosaicity was used to make sure all the observed diffraction intensities were predicted (Table 1). Data previously measured using a standard Si monochromator typically gave mosaicities of 0.05 for HEW lysozyme and 0.35 for human MTAP. Partials were added and treated in the normal way in *Scalepack*. The processing statistics show that the datasets are of good quality (Table 2). The HEW lysozyme dataset shows a rapid increase of R_{sym} *versus* resolution. This is characteristic of the radiation damage that was observed during the experiment, which tends to affect the highest-resolution data first. The R_{sym} of 0.043 in the lowest resolution bin indicates that the strong reflections, least affected by radiation damage, merge together very well. The overall redundancy is low (1.7) because the data collection was stopped after just 23°. In contrast, the dataset from human MTAP has high overall redundancy (3.1) from the large number of frames and a more gradual fall-off of R_{sym} with resolution, indicative of a low-temperature data collection.

3. Discussion

The observed X-ray intensity of 7×10^{10} photons s^{-1} at 100 mA, through a 0.3 mm collimator, compares well with the value of 4×10^{11} photons s^{-1} at 100 mA on the multipole wiggler station F1 at CHESS. This is reflected in the longer exposure times required using the multilayer for a crystal size of about 0.3–0.4 mm^3 (15–20 s). Data from the same proteins and to similar resolution have previously been collected on station F1 using 5 s exposures. The multilayer used in these tests was purchased several years ago and was not optimized for this application. More suitable optics are currently available, offering higher efficiency. These results suggest that a multilayer with an energy bandwidth of 100 eV would be useful for structural studies on proteins with cell dimensions of at least 250 Å on the beamlines at CHESS. It may also be possible to manufacture a multilayer

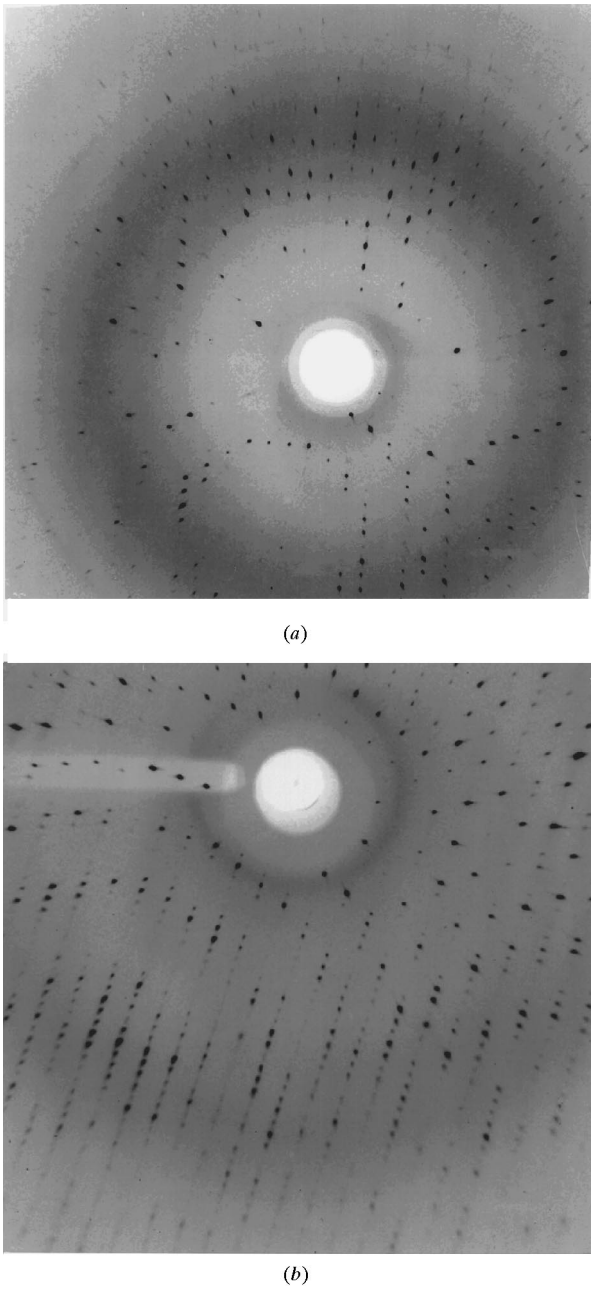

Figure 1
Oscillation images collected on the Princeton 1K CCD detector from (*a*) a crystal of tetragonal HEW lysozyme at room temperature (1° oscillation in 20 s), (*b*) a crystal of human MTAP at 100 K (1° oscillation in 15 s).

offering a selectable bandwidth. Such a device would then offer the crystallographer the possibility of minimizing the exposure time for a protein of given cell dimensions, by using the maximum tolerable bandwidth. The gain in intensity for perfect optics should approximately scale as the bandwidth. When compared with a symmetric Si(111) monochromator, with an intrinsic bandwidth of 1.4×10^{-4}, a 1% bandwidth multilayer offers a 70-fold increase (140-fold for a 2% bandwidth *etc*.) on a broad-band X-ray radiation source, such as a bending or wiggler magnet. A further intensity increase of anywhere from 20–1000 may be possible if the multilayer is used in conjunction with some focusing optics (either a mirror or by bending the multilayer).

The application of multilayers most likely will not be suitable for wavelength-optimized measurements, often used in the initial structure determination of a particular protein (*e.g.* by MAD experiments), where accurate measurement of an often weak anomalous signal is essential. However, there are a host of other studies where fine wavelength resolution is not required. For example, as part of a structural-based drug design project it is often desirable to study a large number of different protein mutants and a variety of protein–inhibitor complexes. The structural information can then be combined with data from other disciplines to provide a more complete picture of an enzyme reaction or ligand-binding mechanism. In these cases the structure of the native protein has usually been determined previously. A dedicated multilayer-based data-collection facility, coupled with high-speed computing and automated refinement procedures, would allow these structures to be determined both rapidly and routinely.

4. Conclusions

A multilayer monochromator has been tested at CHESS, which shows promise for speeding up the rapid structure determination of macromolecules, with cell dimensions of ~250 Å, on bending-magnet synchrotron X-ray sources. Further development by both simulation and testing of a new more perfect optic, as well as developing software to deal precisely with the energy bandwidth, will hopefully improve the data quality and lead to such a device being incorporated on either an existing or new macromolecular crystallography beamline at CHESS.

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