A Modified Mirror Monochromator for Rotation Photography of Large-Unit-Cell Crystals

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A double-mirror monochromator in which one of the mirrors is considerably longer than conventionally employed has been used to collect diffraction data from crystalline tobacco mosaic virus (TMV) protein (unit cell: 224 × 228 × 174 Å). Considerable improvements in speed of data collection are observed over both customary focusing optics and pinhole collimation. Quantitative comparisons are made of the quality of data collected from TMV protein by this method and by the use of nickel-filtered radiation.

Introduction

The collection of diffraction data from crystals with very large unit cells poses severe problems of collimation and monochromation. The requirement for good order-to-order resolution means that if pinhole collimation is used, long exposure times are required as the pinhole must be no larger than about 0.2 mm in diameter for crystals with unit cells of about 200 Å on the edge. This in turn leads to high backgrounds. Arndt (1968) has shown that the most efficient data collection strategy for large-unit-cell crystals is screenless rotation or oscillation photography. In such screenless methods, high backgrounds are particularly problematic since all background radiation is collected by the film.

Consideration of various methods of collimation and monochromation has been given by Arndt & Sweet (1977). As alternatives to pinhole collimation, these authors consider the theoretical performance of both mosaic-crystal monochromators and curved-mirror monochromators. The former device, while giving excellent monochromation, produces a broad beam with a fairly high crossfire and low intensity. The latter is not a monochromator in the same sense as a crystal, providing partial monochromation only, but does produce an intense, well collimated beam which gives cleaner backgrounds than filtered radiation. Where problems of resolution and intensity are particularly severe, it is the only reasonable approach to collimation and monochromation. Monochromation is produced by total external reflexion of the X-rays from a glass mirror (usually nickel coated). The critical angle is of the order of a fraction of a degree and proportional to the X-ray wavelength, so that if, for instance, copper radiation is used, the Kβ line and most of the high-energy white radiation will not be reflected if the glancing angle is kept close to the critical angle for the Ka line (see, for example, Witz, 1969). If the X-rays are reflected from two mirrors set at right angles and bent to approximate to elliptical surfaces, a point focus can be obtained. In practice, the beam is not truly 'focused'. Because of factors such as finite source size and imperfect shape of the reflecting surface, a caustic is obtained, and the width of the 'focused' beam varies little over the distance between the monochromator and film.

This monochromator was first used by Franks (1955) and has since been used regularly in fibre diffraction experiments. It was applied to single-crystal work by Harrison (1968) who described a design for the mirror bender, similar to one then being used in this laboratory in studies on viruses and muscle (H. E. Huxley and K. C. Holmes, unpublished), in which the mirror is bent by the application of equal couples at the ends. This design has been used routinely for data collection from some very large-unit-cell crystals (Winkler, Schutt, Harrison & Bricogne, 1977) and consists of two 6 cm long mirrors placed close to the X-ray source to maximize the aperture of the monochromator. The improvement in aperture of the double-mirror monochromator over pinhole collimation depends on the size of the unit cell, since the intensity obtained with the former is virtually independent of the order-to-order resolution required. Arndt & Sweet (1977) have made theoretical comparisons of the intensities obtainable with the double-mirror monochromator in contrast to optimum pinhole collimation and conclude that the intensities would be roughly equal for a crystal whose unit-cell edge is about 180 Å. Their calculation seems to contain an arithmetical error, and the true cross-over point is about 370 Å. However, since optimum pinhole-collimation conditions are never achieved in practice, the cross-over point would occur at a rather smaller unit-cell size. As yet no quantitative comparisons have been made of data collected by the two methods. For tobacco mosaic virus (TMV) protein crystals (unit cell 224 × 228 × 174 Å), I have found that this design of mirror monochromator gives slightly less intensity than pinhole collimation, although the

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reflexions are much better resolved and the background is cleaner (Fig. 1).

Modification of the monochromator to give high intensity

It has been suggested (for example by Harrison, quoted by Arndt & Sweet, 1977) that the use of the double-mirror monochromator may be extended to smaller-unit-cell crystals if the length of the second mirror (that further from the source) were increased. This is because the aperture of the monochromator depends on the length of the mirrors, the longer mirror subtending a larger angle at the source. There is little to be gained, however, by increasing the length of the first mirror, since this reduces the aperture of the second by placing it further from the source. Using an expression for the aperture of the mirror given by Arndt & Sweet (1977), the aperture of the system of two 6 cm mirrors in practical conditions is $0.7 \times 10^{-5}$ sterad. If the length of the second mirror is increased to 20 cm, the total aperture becomes $1.7 \times 10^{-5}$ sterad. The use of a 30 cm second mirror would give an aperture of $2.1 \times 10^{-5}$ sterad, so that there is little to be gained by using mirrors longer than about 20 cm.

I have tested the practical performance of this modified mirror monochromator by collecting diffraction data to 5 Å resolution from TMV protein crystals using a monochromator consisting of a 6 cm and a 20 cm mirror, both nickel plated, in conjunction with an Arndt–Wonacott rotation camera (Arndt, Champness, Phizackerley & Wonacott, 1973). These data are readily comparable with 5 Å resolution data from the native protein and two heavy-atom derivatives – methyl mercury nitrate (MMN) and $(AuCl_4)^{-}$ – collected by pinhole collimation with a nickel filter (Champness, Bloomer, Bricogne, Butler & Klug, 1976). In their avoidance of excessively long exposure times, these authors accepted data of considerably poorer quality than can normally be obtained from small proteins. Two improvements may be expected from the use of the modified mirror monochromator. Firstly, exposure times should be reduced owing to the increased aperture, and secondly, data quality should be improved owing to the lower background of the films. These aims are to some extent complementary, and some compromise between the two will be necessary depending on the requirements of the experiment.

Fig. 1 shows three rotation photographs of TMV protein collected by (a) the conventional double-mirror monochromator, (b) the modified monochromator described here, and (c) pinhole collimation. The reduced background and improved order to order resolution are very evident in (a) and (b), but note that an improvement in exposure time is obtained by the use of the longer mirror. The increased crystal-to-film distance in case (b) is used to improve the signal-to-noise ratio, since the intensity of the focused beam falls off only slowly with distance while the back-
Fig. 2. Curves of $R$ factor (for symmetry) against $|F|$ for the data sets compared in Table 1. The data points show the $R$ factor calculated for all reflexions in a given amplitude group and the mean value of $|F|$ in that group.
The data presented here were collected using an Elliot GX13 rotating-anode X-ray generator with a minimum foreshortened source size of 0.1 × 0.1 mm. Provided this source size was maintained, Kβ contamination of the doubly reflected beam was not troublesome with either the 20 cm or the 6 cm mirror, presumably because the reflectivity of the nickel surface for Cu Kβ radiation increases only slowly for glancing angles just under the critical angle owing to the high absorption coefficient. If, however, the source size is allowed to increase beyond this minimum, Kβ contamination quickly becomes excessive. This is particularly true in the case of the long mirror because of the greater intrinsic spread of glancing angles, and in this case the requirement for a fine X-ray source is especially stringent. To some extent this difficulty could be overcome by moving the mirror further from the source, but the improvement would be achieved at the expense of loss of intensity.

The use of a second mirror longer than 20 cm would create greater problems of monochromation while giving only a minimal improvement in intensity as noted above. It would appear then that the greatest practical length for the second mirror is about 20 cm, if the double-reflexion system is to be used as a monochromator.

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