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Direct Phase Determination for Macromolecular Crystals Using the Multiple-Diffraction Technique and an In-House X-ray Source

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Abstract

This paper reports the first attempt at using a rotating-anode X-ray source and the multibeam diffraction technique to determine the phases of X-ray reflections from macromolecular crystals. Invariant phases of structure-factor triplets of lysozyme are determined unambiguously *via* four-beam diffractions.

Introduction

The determination of triplet-invariant phases of macromolecular crystals using X-ray multiple diffraction has been demonstrated with synchrotron radiation (Hümmer, Schwegle & Weckert, 1991; Chang, King, Huang & Gao, 1991; Hümmer, Schwegle & Weckert, 1992). It is, however, generally believed that conventional laboratory X-ray sources may not be suitable for obtaining intensity profiles with multiple diffraction and for revealing the phase effect from the multibeam interaction in macro-molecular crystals because the low photon flux of the sources gives weak signals and counting statistics, the poor degree of beam parallelism makes coherent interaction of X-ray wavefields difficult and the untunable wavelength provides no way of avoiding peak overlapping. In this communication, we report efforts towards overcoming these difficulties, success in the detection of multiple diffraction profiles from

lysozyme using a conventional X-ray source and the results of quantitative determination of the invariant phases associated with the multibeam cases studied.

Experimental

Multiple-diffraction experiments were carried out on a four-circle diffractometer (Huber 5042) with a rotating-anode generator (Elliott, GX-21). A hen egg-white lysozyme crystal ($a = b = 78.9$, $c = 38.1$ Å, tetragonal, space group $P4_32_12$) was first aligned for a primary reflection G and then rotated (ψ rotation) around the reciprocal-lattice vector g of the G reflection to bring secondary reflections L and M into position to diffract simultaneously the incident beam. The intensity variation of the primary reflection during the ψ scan is recorded as the multiple-diffraction profile. In order to increase the visibility of this diffraction profile and to improve the angular resolution, we let the direct beam go through a collimation pipe 120 cm long with a pinhole 0.3 mm in diameter at its exit, so that the incident beam hit the crystal sample directly and the beam divergences were 0.04° vertically and horizontally for a point source 0.1 × 0.1 mm from a copper target. A nickel filter was used. In addition, we chose strong four-beam diffractions with intensities at least one order of magnitude higher than the adjacent multiple diffractions, according to intensity calculations based on the kinematical theory (see Moon & Shull, 1964).

To monitor the secondary reflection, we attached an additional vertical axis for the detector onto the 2θ arm; the detector holder can be translated along the direction normal to the diffraction plane and tilted toward the secondary reflected beam. Once the secondary reflection was located by the detector, its Miller indices were identified from the orientation matrix. The detector was then moved onto the diffraction plane to detect the primary reflected beam. The ψ scan for this multiple diffraction was then carried out. To minimize the low-frequency noise coming from the X-ray source, the same ψ scan was repeated at least 30 times over an angular range of about 0.2° at intervals of 0.004°. The counting time at each angular setting was 5 s. The working conditions of the generator were 40 kV and 100 mA. The crystal size was about 0.3 × 0.5 × 0.8 mm.

Results

Multiple-diffraction patterns of the ψ scans are shown for the two centrosymmetrically related four-beam cases $000,550,120,340/000,\overline{550},\overline{120},\overline{340}$ and $000,550,542,102/000,\overline{550},\overline{542},\overline{102}$ in Figs. 1 and 2, respectively. Since 550 and $\overline{550}$ are the primary

reflections, the crystal rotation axis is a twofold axis. The predominant triplet phases δ_3 involved in a four-beam (O,G,L,M) case are $\delta_{\overline{G}} + \delta_L + \delta_{G-L}$ and $\delta_{\overline{G}} + \delta_M + \delta_{G-M}$ (ignoring the correction for anomalous dispersion). In a symmetric four-beam case like those mentioned above, with a twofold axis as the rotation axis, the two predominant phases are identical (Hümmer, Bondza & Weckert, 1991). The predominant phases involved in those two centrosymmetrically related four-beam cases were quantitatively determined following the procedure described by Chang, Tsai & Huang (1993) and by Chang, King, Huang & Gao (1991), where the fact that $\sin \delta_3$ and $\cos \delta_3$ are related, respectively, to the intensities at $\Delta\psi = 0$ and the intensities at half-widths was considered. Curve fitting and least-squares refinements of the position $\Delta\psi = 0$ and peak width were employed. The phases determined from the curve-fitted intensity profiles are $\delta_3 = \delta_{\overline{550}} + \delta_{120} + \delta_{430} = 12 \ (1)°$, $\delta_3 = \delta_{550} + \delta_{\overline{120}} + \delta_{\overline{430}} = -7 \ (1)°$, $\delta_3 = \delta_{\overline{550}} + \delta_{542} + \delta_{01\overline{2}} = -74 \ (12)°$, $\delta_3 = \delta_{550} + \delta_{\overline{542}} + \delta_{0\overline{12}} = 88 \ (10)°$, compared, respectively, with theoretical values of 0, 0, -72 and 72° calculated from the known structure of lysozyme (Diamond, Phillips, Blake & North, 1974; Brookhaven Protein Data Bank entry 1LYZ in the version of 1975). The
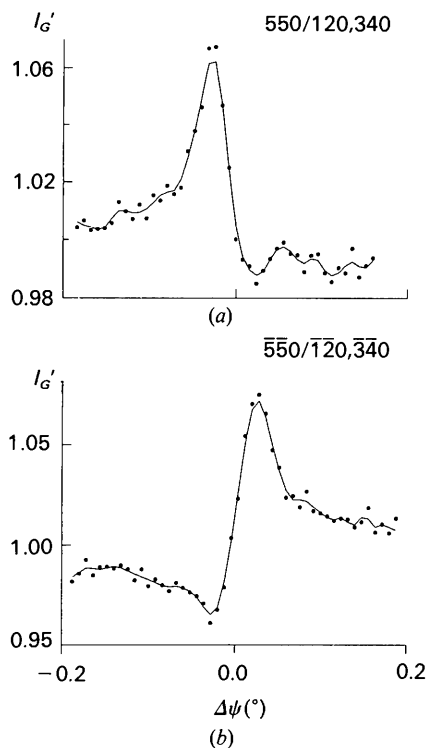

Fig. 1. ψ-scan profiles of lysozyme, $\lambda = 1.54056$ Å. (a) Four-beam case 550/120,340 (OUT), $\delta_3(\exp) \simeq 12°$; (b) four-beam case $\overline{550}/\overline{120},\overline{340}$ (IN), $\delta_3(\exp) \simeq -7°$. The IN and OUT indicate the positions at which the reciprocal-lattice points of the secondary reflections, 120 and 340, are moving towards or away from the Ewald sphere. The scan directions are from left to right.
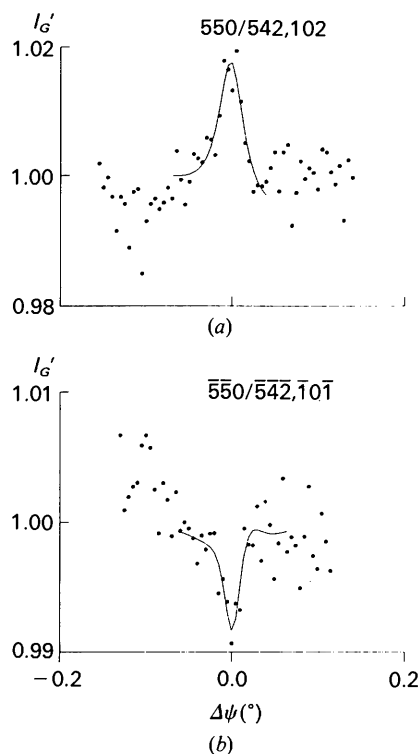

Fig. 2. ψ-scan profiles of lysozyme, $\lambda = 1.54056$ Å. (a) Four-beam case 550/102,542 (OUT), $\delta_3(\exp) \simeq -74°$; (b) four-beam case $\overline{550}/\overline{102},\overline{542}$ (IN), $\delta_3(\exp) \simeq 88°$. The lines show the fitted curves for the phase analysis. The scan directions are from left to right.

phase-angle deviations given above are only indications of the accuracy of the refinement procedure. The overall errors in the determined phase values are about 30° and come mainly from the intensity fluctuation caused by the beam instability and the overlapping of adjacent weak multiple diffractions. It is worth mentioning that, because of the low X-ray power used, the sample crystal did not deteriorate after six months of X-ray exposure.

Concluding remarks

To sum up, we have demonstrated the possibility of using conventional in-house X-ray sources to carry out multiple diffractions for the phase determination of macromolecular crystals. In addition, we have shown that the use of direct-beam optics without a monochromator, the repetition of the ψ scan and the adoption of a vertical axis for the detector have greatly improved the visibility of the multibeam interaction effect on the diffraction intensity. With this improvement, multiple-diffraction experiments for solving the X-ray phase problem in macromolecular crystals are not limited to the use of synchrotron radiation. Moreover, many more useful phases can be obtained in a relatively longer period of time before the crystal deteriorates.

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Disorder and the Molecular Packing of C60 Buckminsterfullerene: a Direct Electron-Crystallographic Analysis

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Abstract

The crystal structure of C_{60} buckminsterfullerene was determined at room temperature by a direct phasing analysis of single-crystal electron-diffraction intensity data. The initial electrostatic potential map is well fit by a regular icosahedron of C atoms but with an average rotational disorder corresponding to $Fm3m$ symmetry. The static appearance of this directly determined map, however, does not refute the notion of uncorrelated molecular positions in the crystal lattice, indicated earlier by nuclear-magnetic-resonance spectroscopy and neutron scattering. Although the direct determination of crystallographic phases is correct, the occurrence of strong axial $h00$ reflections in the electron diffraction patterns appears to be a result of secondary scattering. Correction for this perturbation produces a good fit of the intensities to an isotropic spherical shell of C atoms. In fact, the static appearance of the initial potential map is artificial, owing to the use of only a limited set of phased structure factors in the Fourier transform carried out after the ab initio structure analysis.

Introduction

Since the discovery of C_{60} buckminsterfullerene (Kroto, Heath, O'Brien, Curl & Smalley, 1985), considerable effort has been made to characterize the molecular packing in the solid state of this unusual