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Description of Overall Anisotropy in Diffraction from Macromolecular Crystals

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Abstract

Simple formulations are described for evaluating overall anisotropy of the kind sometimes found in diffraction from crystals of macromolecules. The models correspond to whole-body anisotropic vibration of unit cells or of asymmetric macromolecular units that internally also undergo local isotropic atomic motions. These procedures have been implemented in programs that (1) use the structure-factor components from individual molecules to evaluate the anisotropy, (2) use existing F_c data to determine anisotropic parameters for the unit cell, and (3) use expected intensity values from unit-cell contents for unknown structures. The methods have been applied in refinement of the structures of myohemerythrin and other proteins and this led to improved R values and more readily interpreted difference maps.

Introduction

It occasionally happens that diffraction from a molecular crystal is appreciably anisotropic in its overall dependence on scattering angle. Such anisotropy is sometimes of sufficient magnitude that the limiting extent of measurable data differs in different directions. Examples from macromolecular crystallography include the nucleosome core particle where diffraction extends to about 5, 6 and 8 Å spacings along orthogonal axes (Finch, Brown, Rhodes, Richmond, Rushton, Lutter & Klug, 1981), a bacteriophage 434 repressor-operator complex where the data extend to about 3 Å spacings in the directions of protein-DNA rods whereas the limit is about 4 Å in other directions (Anderson, Ptashne & Harrison, 1984), a $\gamma\delta$ resolvase crystal that diffracts to 4 Å along the hexagonal axis of the lattice but only to 7 Å in perpendicular directions (Abdel-Meguid, Grindly, Templeton & Steitz, 1984) and, from our own work, myohemerythrin for which data were measurable to a limit of 1·3 Å along an axis that parallels the α helices of the structure as compared with a limit of 1·7 Å spacings orthogonally where lattice contacts are sparse. Even when the limits of diffraction are not encountered in an experiment, significant departures from overall isotropy can sometimes be detected.

In the case of small molecules where data extend to high angles, any overall anisotropy that exists is naturally included in the individual anisotropic temperature parameters of the refined atomic model. On the other hand, limitations in the extent of diffraction from macromolecules often dictate that such crystal structures be refined isotropically in order to preserve a favorable observation-to-parameter ratio. If substantial overall anisotropy is present, the isotropic model will necessarily be inadequate near the diffraction limit. However, a simple description of this general anisotropy can appreciably improve the agreement between observation and calculation. In addition to its impact on refinement, this description gives information about the anisotropic motion of molecules in the lattice and it might also be useful in other aspects of crystallographic analysis. We present here a description of overall anisotropy and provide procedures for evaluating its parameters. The methods have been tested with data from crystals of myohemerythrin and other proteins.

Theoretical formulation

There are many possible causes for overall anisotropy in diffraction, and a mathematical description of the effect will depend on the particular physical basis. Our main objective here is a simple formulation that captures the essential features of the observations. This can be achieved with an overall anisotropic increment in thermal parameters. Such a model corresponds to ascribing a whole-body anisotropic vibration to macromolecular units that internally also undergo local isotropic atomic motion.

The structure factor F for a model with overall anisotropy applied to individual asymmetric units is given by

$$F(\mathbf{h}) = \sum_{k=1}^{N_s} \sum_{j=1}^{N_a} f_j(s) \exp(-B_j s^2) \times \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_{j,k}) \exp(-\mathbf{h}^T \Delta\boldsymbol{\beta}_k \mathbf{h}). \quad (1)$$

Here $\mathbf{h}(h, k, l)$ is the vector of reciprocal-lattice

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indices; $s = \sin(\theta)/\lambda$; f_j, B_j and $\mathbf{x}_j(x, y, z)$ are respectively the atomic scattering factor, isotropic thermal parameter and fractional atomic position vector for the jth of N_a atoms in the asymmetric unit; and $\mathbf{x}_{j,k}$ and $\mathbf{\Delta\beta}_k$ refer to the atomic coordinates and overall thermal-parameter increments for the kth of N_s symmetry equivalents. If the symmetry operation that relates coordinates of the kth unit to those of the fundamental unit, $\mathbf{x}_{j,0}$, is given by

$$\mathbf{x}_{j,k} = \mathbf{S}_k\mathbf{x}_{j,0} + \mathbf{t}_k,$$

then the incremental thermal ellipsoid as oriented in the kth unit is

$$\mathbf{\Delta\beta}_k = \mathbf{S}_k\mathbf{\Delta\beta}_0\mathbf{S}_k^T$$

in relation to the ellipsoid of the fundamental unit, $\mathbf{\Delta\beta}_0$. The symmetry operators of rotation $\mathbf{S}_k$ and translation $\mathbf{t}_k$ are normally those of the particular space group, but these could also be extended to include non-crystallographic symmetry.

It may happen that one cannot readily ascertain the orientation of the thermal ellipsoid of the macromolecular asymmetric unit. In this event it is useful to consider an overall anisotropic motion of the entire unit cell in the lattice. The structure factor for this model of whole-cell anisotropy is

$$|F(\mathbf{h})| = |G(\mathbf{h})| \exp(-\mathbf{h}^T\overline{\mathbf{\Delta\beta}}\mathbf{h}) \qquad (2)$$

where $G(\mathbf{h})$ is the isotropic structure factor:

$$G(\mathbf{h}) = \sum_{k=1}^{N_s}\sum_{j=1}^{N_a} f_j(s) \exp(-B_j s^2) \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_{j,k}).$$

The anisotropic parameter in this case is denoted by $\overline{\mathbf{\Delta\beta}}$ since it corresponds to the orientational average of all symmetry equivalents. This formulation obviously applies to the situation where structure factors from an isotropic model are to be compared with observations that might include anisotropy.

It can also be useful to evaluate anisotropy at a stage when an atomic model is not yet available. Provided that the contents of the asymmetric unit are known, the anisotropy in this case can be expressed as

$$|F(\mathbf{h})|^2 = E(\mathbf{h}) \exp(-2\mathbf{h}^T\bar{\mathbf{\beta}}\mathbf{h}) \qquad (3)$$

where the expected value for the squared structure-factor modulus (normalized intensity) in the absence of atomic displacements (Wilson, 1942) is given by

$$E(\mathbf{h}) = N_s \sum_{j=1}^{N_a} f_j^2(s).$$

Here the overall anisotropic temperature parameter also incorporates the isotropic component and, as for (2), these parameters are averages that pertain to the whole unit cell. Thus, there are restrictions on the elements of these thermal-parameter tensors, $\overline{\mathbf{\Delta\beta}}$ and $\bar{\mathbf{\beta}}$, that reflect the point symmetry of the Laue group (Prince, 1982).

Computational aspects

While it is convenient for diffraction calculations to express anisotropic thermal parameters in the form of the dimensionless $\mathbf{\beta}$'s, as above, these measures obscure the physical meaning. The isotropic parameters are directly related to mean-square atomic displacements, $B = 8\pi^2\overline{u^2}$, and thus it is useful to express the anisotropic parameters on the same scale. The correspondence of elements in a $\mathbf{\beta}$ representation to those in a $\mathbf{B}$ representation is $\beta_{ij} = \frac{1}{4}a_i^* a_j^* b_{ij}$ where $\mathbf{B}$ is in a general, possibly non-orthogonal, coordinate frame and a_i^* is the length of a reciprocal-lattice edge. Thus, the exponent of anisotropy in (1) expands to

$$\mathbf{h}^T\mathbf{\Delta\beta}\mathbf{h} = \tfrac{1}{4}(b_{11}a^{*2}h^2 + 2b_{12}a^*b^*hk + 2b_{13}a^*c^*hl + b_{22}b^{*2}k^2 + 2b_{23}b^*c^*kl + b_{33}c^{*2}l^2). \qquad (4)$$

We have developed three computer programs to evaluate overall anisotropy in macromolecular crystals. Each finds the least-squares fit of the elements of the anisotropic tensor to the observed structure-factor moduli $|F_o|$. One program compares the $|F_o|^2$ data to expected values given by (3). A second program implements (2) to bring the $|F_c|$ values calculated from an isotropic model into optimal agreement with anisotropic $|F_o|$ observations. In both of these programs, symmetry restrictions on tensor elements must be taken into account (*e.g.* $b_{12} = b_{23} = 0$ for monoclinic; $b_{11} = b_{22}$ and $b_{13} = b_{23} = 0$ for tetragonal Laue group 4). The third program treats the more general case given by (1). This analysis requires that the separate real and imaginary components, A and B, be available for each symmetry element. Thus, for this case it was also necessary to modify the restrained refinement program *PROLSQ* (Hendrickson & Konnert, 1980; Hendrickson, 1985) to provide input for the least-squares evaluation and to apply the resulting anisotropic factor in succeeding cycles of refinement. Typically, we re-evaluate the anisotropy every few cycles.

Although the formulations given by (1) and (2) correspond to a physical model that has whole-body anisotropic motion superimposed on isotropic internal vibrations of individual atoms, the data from typical experiments do not suffice to distinguish these effects completely. The distinction between the average of isotropic B values and the average anisotropic level (from the trace of $\mathbf{\Delta\beta}$) is arbitrary. In our computations, the separation is set by the average B in the isotropic refinement preceding the anisotropic fitting.

Applications

The analysis of overall anisotropy presented here was motivated by our need to account for anisotropy in diffraction from myohemerythrin crystals during refinement of the structure. Myohemerythrin from the

sipunculan worm *Themiste zostericola* crystallizes from ammonium sulfate in space group $P2_12_12_1$ with $a=41{\cdot}66$, $b=80{\cdot}17$ and $c=37{\cdot}82$ Å. The structure was initially determined by multiple isomorphous replacement at 5·5 Å resolution (Hendrickson, Klippenstein & Ward, 1975) and this result was extended to 2·8 Å resolution by model-resolved anomalous phasing. During data collection for a high-resolution analysis we noticed that whereas data in the direction of b^* could be measured to 1·3 Å spacings, in the perpendicular directions (a^*c^* plane) diffracted intensities had already fallen to this level for reflections corresponding to 1·7 Å spacings. Consequently, data measurements were restricted to an ellipsoid of reciprocal space specified by semi-axes of (1/1·7) Å^{-1} along a^* and c^* and by (1/1·3) Å^{-1} along b^*.

Initially, we attempted to account for the myohemerythrin anisotropy by 'correcting' the observed data. The new 3·0–1·7/1·3 Å data set was scaled to the existing ∞–2·8 Å set by a factor of $\exp\{\frac{1}{4}[6{\cdot}6(a^{*2}h^2+c^{*2}l^2)]\}$. These corrected data were then used in the early stages of refinement. A later analysis of another data set (∞–2 Å) showed that the ∞–2·8 Å data set could not be isotropic as had been assumed, and this provoked the analysis described here. Structure factors calculated from a model that had been refined against the corrected data were compared against uncorrected measurements to deduce overall anisotropic parameters. The results are shown in Table 1.

Table 1. *Overall anisotropic thermal parameters* (Å^2) *for myohemerythrin*

	Formulation (3) $\langle F\rangle$ $\overline{\mathbf{B}}$	Formulation (2) F_c $\overline{\Delta\boldsymbol{\beta}}$	Formulation (1) A_c, B_c $\Delta\boldsymbol{\beta}$
b_{11}	23·5	5·8	5·8
b_{12}	—	—	−0·7
b_{13}	—	—	0·5
b_{22}	13·1	−9·0	−9·0
b_{23}	—	—	−0·5
b_{33}	20·8	3·3	3·3
Average B_{iso}	—	22·9	22·9
R(anisotropic)	0·424	0·164	0·163
Isotropic B	18·4	−1·1	−1·1
R(isotropic)	0·447	0·230	0·230

The data used in these fittings included all reflections greater than $2\sigma_I$ and ranged from 10 to 1·3 Å in Bragg spacings. Least-squares fittings were made to each of the formulations of overall anisotropy as described in the text. Respectively, these fitted the expected structure-factor moduli $\langle F\rangle$, isotropic calculated structure factors F_c, and the structure-factor contributions A_c and B_c of the asymmetric units to the observed diffraction data. Structure factors for these fittings were calculated after refinement cycle XVIII.3. Thermal parameters are given in B values that relate to the respective β values according to (4). The average B_{iso} cited here pertains to protein atoms only. The isotropic B and associated R value were calculated to provide a basis for comparing the improvement in the model which would not be biased by the current average B.

All three procedures that we have presented for describing overall anisotropy produced similar values for myohemerythrin. This model of anisotropy appreciably improved the agreement factor from $R=0{\cdot}23$ to $R=0{\cdot}16$. Relative to the b^* direction the thermal parameters are large along a^* and c^*, and the molecular ellipsoid is nearly aligned with the crystal axes. This behavior is consistent with two aspects of the structure that are illustrated in Fig. 1 which shows a projection of the α-carbon backbone down the b axis onto the xz plane. Firstly, there are very few lateral lattice contacts of a unique molecule with its neighbors whereas molecules at ±0·5 in y (not shown for clarity) make several contacts with the central molecular. Moreover, nearly all of the lateral contacts that do exist involve the relatively mobile *N*-terminal arm. Secondly, the four-helix bundle of myohemerythrin is aligned along the b axis. It may be that the normal modes of myohemerythrin permit larger-scale displacements of the helices laterally with respect to one another than of residues axially within the helices.

We have also evaluated anisotropy in protein crystals for which there is no striking differential fall off in diffracted intensity. Results from applying the program that implements (2) to compare F_o with F_c have led, in these cases, to some improvement in R (0·001 to 0·007) with anisotropic $\Delta\boldsymbol{\beta}$ elements of 2 Å^2 or less. In the case of bovine pancreatic trypsin inhibitor, values of $b_{11}=-1{\cdot}69$, $b_{22}=2{\cdot}05$ and $b_{33}=-0{\cdot}26$ Å^2 were obtained for the isotropic model from the refinement reported by Yu, Karplus & Hendrickson (1985), and this reduced R from 0·152 to 0·145. The improved agreement in this application of overall anisotropy compares favorably with an R of 0·143 obtained in the individual three-parameter anisotropic model (Yu *et al.*, 1985). In the case of erabutoxin (Smith, Corfield, Hendrickson & Low, 1987), R was reduced from 0·163 to 0·159 with b_{11}, b_{22}

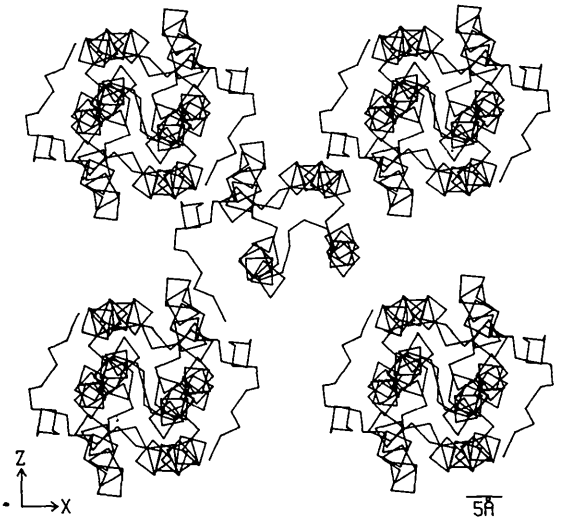

Fig. 1. Projection onto the xz plane of neighboring molecules in $P2_12_12_1$ crystals of myohemerythrin. The schematic diagram for myohemerythrin is the α-carbon connectivity. For clarity molecules at $y\pm0{\cdot}5$ above and below the central unit were not drawn. Notice the absence of interactions between the central molecule and those in the lower-right-hand corner.

and b_{33} of $-1{\cdot}54$, $0{\cdot}62$ and $0{\cdot}92$ Å^2 respectively (final values were $-1{\cdot}42$, $0{\cdot}39$ and $1{\cdot}03$ Å^2). Treatment of anisotropy in this way made difference maps less noisy and this facilitated the further interpretation of the structure.

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General Density Function Corresponding to X-ray Diffraction with Anomalous Scattering Included

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Abstract

The generalized density function that is the Fourier transform of X-ray diffraction as observed when anomalous scattering occurs is described. This is a complex function in contrast to the purely real electron-density function that pertains when only the 'normal' Thomson scattering component is present. The imaginary component of this general density function produces an image of the anomalous scattering centers and is more accurate than the Kraut approximation commonly used in macromolecular crystallography to produce such images.

Introduction

The Fourier transform of the electron-density distribution in an atom yields the normal atomic scattering factor for X-rays. Conversely, a Fourier synthesis of the structure factors from a crystal composed of such normal scatterers gives back the true electron-density function, $\rho(\mathbf{x})$. This 'normal' situation pertains if the scattering from each point is directly proportional to that from a free electron. In reality, the scattering process can involve resonance with the natural frequencies of bound electrons and this leads to additional phase-shifted contributions - the anomalous scattering (James, 1948). A Fourier synthesis of the structure factors from a crystal that includes anomalous scatterers does not produce the true electron-density distribution, which is real and non-negative, but by analogy we can define a general density function, $\rho^*(\mathbf{x})$, as the Fourier transform of the actual X-ray diffraction rather than just the normal scattering component. This function is complex and the imaginary component depends only on the anomalous scattering centers. The Bijvoet-difference Fourier synthesis proposed by Kraut (1968), a function which has proved useful in macromolecular crystallography, is an approximation of the true imaginary component (Chacko & Srinivasan, 1970). In this paper we examine the properties of the general density function, test approximations with simulated diffracted data, and discuss applications with experimental data.

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