TREATMENT OF LOW ANGLE X-RAY DATA FROM PLANAR AND CONCENTRIC MULTILAYERED STRUCTURES

A. E. BLAUROCK *and* C. R. WORTHINGTON

From the Department of Physics and the Biophysics Research Division, University of Michigan, Ann Arbor

ABSTRACT Low angle X-ray diffraction can be recorded from planar and concentric multilayered (biological) structures. In order to proceed with the X-ray analysis the relation between the observed intensities and the Fourier transform of the unit cell is required. This relation is derived for planar structures such as retinal rods, mitochondria, and collagen; and also for nerve myelin.

INTRODUCTION

The structures considered here have limited order in contrast to crystals which have three dimensional order. In an X-ray analysis of a crystal, the treatment of X-ray data does not present any problem, for diffraction formulas are available. The relation between the integrated intensities and the Fourier transform of the unit cell of a crystal has been derived (see James, 1948). However, if the structures have limited order, then the situation is different and a separate study is needed; the crystal procedures do not, in general, apply. In this paper we consider the treatment of low angle X-ray data from such structures; the analysis is restricted to structures with large unit cells which have either linear or radial repeats. Such structures have a widespread occurrence in biology.

Low angle X-ray diffraction has been recorded from various lipoprotein membrane structures such as retinal rods (Finean et al., 1953), mitochondria (Worthington, 1960) and nerve myelin (see Finean, 1962), and other fibrous protein structures such as collagen. Retinal rods, mitochondria, and collagen are examples of planar multilayered structures whereas nerve myelin is an example of a concentric multilayered structure. Discrete low angle X-ray diffraction is observed because these structures have well-defined repeating units; linear repeats for the planar structures and a radial repeat for nerve myelin. The linear repeats are as follows: retinal rods have a disc-to-disc distance of about 300 A, mitochondria have a cristae-to-cristae distance of about 300 A, and collagen has a fiber axis repeat of 640 A. The radial repeat in nerve is about 170 A.

The low angle X-ray spacings give the repeating distance of the structure while the X-ray intensities contain information on the large-scale structure of the unit cell. By large-scale structures we have in mind uniform electron density distributions confined to a comparatively small number of layers within the unit cell. In order to test whether some proposed model might fit the observed X-ray data, a relation between the integrated intensities and the Fourier transform of the unit cell is required.

Statement of Problem. A one dimensional variation of structure $t(a)$ is pertinent in the description of layered structures; $t(a)$ is the electron density which repeats at intervals of d along a. We define $t_c(a)$ as the electron density of a single unit cell of size d. The Fourier transform of $t_c(a)$ is denoted $T(a^*)$:

$$T(a^*) = \int_0^d t_c(a) \exp(i2\pi a a^*)\, da, \tag{1}$$

where a, a^* are direct and reciprocal space coordinates.

In an X-ray experiment on layered structures the integrated intensity $I(h)$ of a discrete series of reflections is recorded. The diffraction maxima occur at $a^* = h/d$, h an integer. The Fourier series representation of $t_c(a)$ is

$$t_c(a) = 1/d \sum_{-h}^{+h} T(h) \exp(-i2\pi h a/d). \tag{2}$$

The relation between the Fourier transform of the unit cell and the integrated intensities is written

$$I(h) \alpha \Delta(h) |T(h)|^2 \tag{3}$$

where α is the proportional sign.

In order to obtain $|T(h)|$ from the intensity $I(h)$, the value of $\Delta(h)$ is required. In this paper we derive the function $\Delta(h)$ for planar and concentric multilayered structures.

We restrict the analysis to structures which have rotational symmetry about the fiber axis and uniform cylindrical radius. The cylindrical structure has volume V, radius R, and length L. We use cylindrical coordinates z, r, ϕ; in the planar structures $t(a)$ occurs along the fiber axis (z), whereas in the concentric structures $t(a)$ occurs along the radial direction (r).

Planar Multilayered Structures. The electron density of the three dimensional structure of volume V is denoted $g(\mathbf{r})$. The Fourier transform of $g(\mathbf{r})$ is $G(\mathbf{r}^*)$:

$$G(\mathbf{r}^*) = \int_V g(\mathbf{r}) \exp(i2\pi \mathbf{r}\cdot\mathbf{r}^*)\, dv \tag{4}$$

Vector $\mathbf{r}$ has cylindrical coordinates r, z, ϕ and element of volume dv is given by $dv = dz\, ds$ where $ds = r\, dr\, d\phi$. Reciprocal vector $\mathbf{r}^*$ has cylindrical coordinates

r^*, z^*, ϕ^* in reciprocal space and element of volume $dv^* = dz^*\, ds^*$, where $ds^* = r^*\, dr^*\, d\phi^*$. The structure has rotational symmetry about the fiber axis and constant radius R. The electron density $g(\mathbf{r})$ becomes

$$g(\mathbf{r}) = \rho(z)u(r), \tag{5}$$

where $u(r) = 1$ when $r \leq R$, $u(r) = 0$ when $r > R$. We identify $\rho(z)$, the one dimensional electron density variation along the fiber axis, with $t(a)$ and write

$$g(\mathbf{r}) = t(a)u(r). \tag{6}$$

Substitution of equation (6) into equation (4) allows the Fourier transform of the structure $G(\mathbf{r}^*)$ to be written

$$G(\mathbf{r}^*) = T(a^*)\Phi(a^*)\, U(r^*) \tag{7}$$

$T(a^*)$ is the Fourier transform of $t_c(a)$ and is given by equation (1). $\Phi(a^*)$ is the interference function due to N unit cells; the length of the structure along the fiber axis is $L = Nd$. $\Phi(a^*)$ is given by

$$\Phi(a^*) = \sin N\pi a^* d / \sin \pi a^* d. \tag{8}$$

$\Phi(a^*)$ has maxima when $a^* = h/d$, h is an integer. $U(r^*)$ is the transverse shape transform of the structure and is given by

$$U(r^*) = \pi R^2 J_1(2\pi R r^*)/\pi R r^*. \tag{9}$$

$U(r^*)$ has rotational symmetry about a^*, that is, it is independent of ϕ^*.

We note that equations (1), (7), (8), and (9) were first given by Bear and Bolduan (1950) in reference to a smooth cylinder model for collagen.

The integrated intensity can be derived from an integration in reciprocal space (James, 1948). A geometric picture is helpful in following the derivation. In the case of parallel monochromatic X-radiation a single sphere of reflection which is tangent to the a^* axis, the fiber axis, intersects the intensity transform $|G(\mathbf{r}^*)|^2$. The intensity transform consists of a series of discs spaced at intervals of $a^* = 1/d$ along a^*. Let the discs have effective radius w^*. The intensity recorded depends on the geometry of the intersection.

Consider the case of divergent monochromatic X-radiation, ε is the half angle of the cone of divergence. The sphere of reflection, one point of which is fixed at the origin of the reciprocal space, is effectively rotated through an angle ε from the cone axis. The X-ray diffraction intensity is then expressed as an integral.

From diffraction theory (James, 1948) the integrated intensity $I(h)$ can be written

$$I(h) \alpha (\sin 2\theta)^{-1} \int_{\varepsilon} |G(\mathbf{r}^*)|^2\, dv^*, \tag{10}$$

where α, the proportional sign, is used as we neglect constant factors (see James, 1948), and also neglect the polarization factor as only small angles of diffraction

are considered. θ is the Bragg angle and $\sin 2\theta \approx h\lambda/d$. The term $\sin 2\theta$ arises from the geometry (it appears as a result of expressing the element of divergence in terms of the element of volume in reciprocal space). We need to evaluate the integral (10). $|T(h)|^2$ shows only a slow variation in reciprocal space and hence equation (10) may be written

$$I(h) \alpha (\sin 2\theta)^{-1} |T(h)|^2 \int_{\epsilon} \Phi^2(a^*) U^2(r^*) dv^*. \tag{11}$$

The integral containing the interference function can be approximately written

$$\int_{-\infty}^{+\infty} \Phi^2(a^*) da^*. \tag{12}$$

Hence integral (12) can be evaluated and is a constant for all h.
The remaining integral is

$$\int_{\epsilon} U^2(r^*) ds^*. \tag{13}$$

The limits of integral (13) depend on the size of the discs and the divergence of the X-ray beam. The evaluation of equation (11) using equations (12) and (13) and use of equation (3) gives the function $\Delta(h)$. The value of $\Delta(h)$ depends on the size of the disc and the divergence of the X-ray beam.

There are two special cases, (*a*) and (*b*). (*a*) The structure has large transverse size (R is about 1μ or larger) and the X-ray beam has a moderately large divergence. The integral (13) can be considered as having the limits of $\pm\infty$, that is, $0 \geq \phi \geq 2\pi$, $0 \geq r < \infty$ and hence $\Delta(h) = 1/h$. This is the well-known case of one dimensional fibrous crystallites. (*b*) The structure has a small transverse size and the X-ray beam has small divergence. In this case integral (13) has finite limits, and Tomlin and Worthington (1956) give $\Delta(h) = 1$.

In the general case of moderate transverse size and moderate X-ray beam divergence the function $\Delta(h)$ has values intermediate between those of (*a*) and (*b*), that is $1/h \leq \Delta(h) \leq 1$.

Concentric Multilayered Structures. Consider the structure of nerve myelin composed of N concentric layers of thickness d wrapped around an axon of radius a_0. The outside radius of the nerve is $R = a_0 + Nd$ and L is the over-all length.

We assume uniform electron densities within layers; using cylindrical coordinates, the electron density $g(\mathbf{r})$ of the three dimensional structure can be written

$$g(\mathbf{r}) = t(r)k(z), \tag{14}$$

where $t(r)$ is the electron density along the radial direction and $k(z)$ refers to unit variation along the z-axis, the fiber axis. The Fourier transform of the nerve fiber

$G(\mathbf{r}^*)$ is given by equation (4); substitution of equation (14) into equation (4) gives

$$G(\mathbf{r}^*) = F(r^*)\,K(z^*), \tag{15}$$

where

$$K(z^*) = L \sin \pi Lz^*/\pi Lz^*, \tag{16}$$

and

$$F(r^*) = \int_0^R 2\pi r t(r)\, J_0(2\pi r r^*)\, dr. \tag{17}$$

The zero order Bessel function can be written

$$J_0(2\pi r r^*) \approx \pi^{-1}(rr^*)^{-1/2} \cos(2\pi r r^* - \pi/4). \tag{18}$$

The approximation is valid for the range of r and r^* considered here, an error of less than 1% is introduced (if $a_0 \geq 2d$). Hence, using the exponential form of equation (18) we have

$$F(r^*) = 2(r^*)^{-1/2} \int_0^R (r)^{1/2} t(r) \exp[i(2\pi r r^* - \pi/4)]\, dr. \tag{19}$$

Note that if $r = a_0 + (n-1)d + a$, n an integer, then $dr = da$. Redefining the function t to be zero when $r < a_0$, and $t(a)$ when $r \geq a_0$, then

$$F(r^*) = 2\gamma(r^*)(r^*)^{-1/2} \sum_{n=1}^{N} \tau_n(r^*) \int_0^d [a_0 + (n-1)d + a]^{1/2} t(a) \exp(i2\pi a r^*)\, da, \tag{20}$$

where

$$\gamma(r^*) = \exp[i(2\pi a_0 r^* - \pi/4)], \tag{21}$$

and

$$\tau_n(r^*) = \exp[i2\pi(n-1)\, dr^*]. \tag{22}$$

From equations (19) and (22) the condition for $F(r^*)$ to have sharp diffraction maxima is given by $r^* = h/d$, h is an integer; this is essentially the same condition as for the planar structures to have diffraction maxima. Equation (20) may be expressed in an approximate form by use of the expansion

$$[a_0 + (n-1)d + a]^{1/2} \approx [a_0 + (n-1)d]^{1/2}\{1 + a/2[a_0 + (n-1)d]^{-1}\}, \tag{23}$$

which can be used provided $a_0 + (n-1)d > a$. This inequality is satisfied in published electron micrographs of nerve myelin. For example $a_0 \approx 50$ units and $d \approx 1$ unit in the electron micrograph of the cross-section of a small nerve preparation shown by Finean (1962). Hence $F(r^*)$ can be written

$$F(r^*) = (r^*)^{-1/2}[A(r^*)T(r^*) + B(r^*)\beta(r^*)], \tag{24}$$

where

$$A(r^*) = 2\gamma(r^*) \sum_{n=1}^{N} \tau_n(r^*)[a_0 + (n-1)d]^{1/2},$$

$$B(r^*) = \gamma(r^*) \sum_{n=1}^{N} \tau_n(r^*)[a_0 + (n-1)d]^{-1/2}, \tag{25}$$

and

$$\beta(r^*) = \int_0^d at(a) \exp(i2\pi ar^*)\, da, \tag{26}$$

and

$$T(r^*) = \int_0^d t(a) \exp(i2\pi ar^*)\, da. \tag{27}$$

We note that equation (27) has the same form as equation (1). The correction term $B(r^*)\ \beta(r^*)$ is small and is neglected. Hence $F(r^*)$ can be written

$$F(r^*) = (r^*)^{-1/2} A(r^*) T(r^*). \tag{28}$$

From equations (10) and (15) the integrated intensity $I(h)$ can be written

$$I(h) \alpha (\sin 2\theta)^{-1} \int_\epsilon K^2(z^*)\ |F(r^*)|^2\, dv^*. \tag{29}$$

As before a geometric picture is helpful in following the derivation of the X-ray diffraction intensity. The intensity transform $|G(\mathrm{r}^*)|^2$ consists of a series of rings centered about the origin and confined to the equatorial plane at right angles to z^*, the fiber axis. The sphere of reflection intersects these rings.

We need to evaluate integral (29). From equation (16) $K^2(z^*)$ is confined to a small neighborhood of the equatorial plane and the integral containing $K^2(z^*)$ can be approximately written

$$\int_{-\infty}^{+\infty} K^2(z^*)\, dz^*. \tag{30}$$

Hence integral (30) can be evaluated and is a constant for all h. The remaining integral is

$$\int_\epsilon |F(r^*)|^2\, ds^*, \tag{31}$$

and for a certain beam divergence ε then at values of $r^* = h/d$

$$\int_\epsilon |F(r^*)|^2\, ds^* \alpha\ h\ |F(h)|^2. \tag{32}$$

Noting that $\sin 2\theta \approx h\lambda/d$, the intensity $I(h)$ can be written

$$I(h) \alpha\ |F(h)|^2. \tag{33}$$

We note that Whittaker (1955) has derived the relation between the observed in-

tensities and the Fourier transform of a cylindrical lattice structure composed of discrete atoms. Whittaker's relation is in agreement with equation (33).
From equations (3), (28), and (33) the function $\Delta(h)$ is given by

$$\Delta(h) = 1/h \tag{34}$$

The absolute value of $T(h)$ is given by

$$|T(h)| \alpha [hI(h)]^{1/2}. \tag{35}$$

DISCUSSION

The general expression for the integrated intensity $I(h)$ is given by equation (3). In order to proceed with the X-ray analysis the function $\Delta(h)$ must be correctly chosen. Only a few attempts have been made to analyze planar structures; values of $\Delta(h)$ used are as follows:

Collagen, $\Delta(h) = 1$ (Tomlin and Worthington, 1956)
Mitochondria, $\Delta(h) = 1$ (Worthington, 1960)
Elastoidin, $\Delta(h) = 1$ (McGavin, 1962)

The case of mitochondria is more correctly an intermediate case, for mitochondria (and also retinal rods) have moderate transverse size and fine collimated beams are used to record the diffraction. The case of collagen calls for comment. If a smooth cylinder model is an appropriate choice for collagen then $\Delta(h) = 1$. However Bolduan and Bear (1951) have shown that, in X-ray patterns of dry collagen, the effective disc radius increases with increasing h. This complicates the analysis and more correctly the function $\Delta(h)$ remains undetermined.

There is some difficulty in recording sufficient data to ensure adequate resolution of a Fourier synthesis; resolution $\approx d/2h_0$ where h_0 is the largest order of diffraction recorded. Many factors determine the value of h_0 but the function $\Delta(h)$ also influences the number of orders recorded. When the function $\Delta(h) = 1$ then a larger value of h_0 is expected. In the case of collagen up to 25 orders can be recorded (Tomlin and Worthington, 1956). When the function $\Delta(h) = 1/h$, as in the case of nerve, a smaller value of h_0 is to be expected.

The structure of nerve myelin has often been studied for it is thought to represent a model structure for membranes. Current membrane models have protein and lipid layers; in a repeat of nerve myelin there are two lipid layers interspaced with protein (Finean, 1962). In discussing membrane structure only one dimensional variation is considered, either along the fiber axis as in retinal rods, or along a radial direction as in nerve myelin. By swelling nerve myelin, and assuming a center of symmetry, Finean (1962), Moodie (1963), and Finean and Burge (1963) have chosen a set of phases for the Fourier transform $F(r^*)$. Finean (1962) and Finean and Burge (1963) then compute a Fourier series for nerve

$$1/d \sum_{-h}^{+h} \pm [I(h)]^{1/2} \cos 2\pi ha/d. \tag{36}$$

From equation (33) Fourier series (36) contains the coefficients $F(h)$ instead of $T(h)$, hence the Fourier series (36) does not refer to $t(a)$, the one dimensional variation of structure along a radial direction in nerve myelin.

In our analysis the Fourier series for $t(a)$ is given by equation (2) and uses the coefficients $T(h)$ defined by equation (35). If we assume that the unit cell of nerve has a center of symmetry then the Fourier series for $t_c(a)$ can be expressed as

$$t_c(a) \propto 1/d \sum_{-h}^{+h} \pm [hI(h)]^{1/2} \cos 2\pi ha/d. \tag{37}$$

We intend to describe the consequence of using the present theory in analyzing low angle X-ray data from nerve myelin in a future publication.

In summary we have derived from first principles the relation between the observed intensities and the Fourier transform of the one dimensional variation of electron density along either the fiber axis in the case of planar structures or along the radial direction in the case of nerve. This relation involves the function $\Delta(h)$ which has the value $1/h$ for nerve myelin and ranges from $1/h$ to 1 for planar structures.

This work was aided by United States Public Health Service Grant GM 09796. A. E. Blaurock is a National Science Foundation Cooperative Fellow.

Received for publication 27 September 1965.

REFERENCES

BEAR, R. S., and BOLDUAN, O. E. A., 1950, *Acta Cryst.*, **3**, 236.
BOLDUAN, O. E. A., and BEAR, R. S., 1951, *J. Polymer Sc.*, **6**, 271.
FINEAN, J. B., 1962, *Circulation*, **26**, 1151.
FINEAN, J. B., and BURGE, R. E., 1963, *J. Mol. Biol.*, **7**, 672.
FINEAN, J. B., SJÖSTRAND, F. S., and STEINMAN, E., 1953, *Exp. Cell. Research*, **5**, 557.
JAMES, R. W., 1948, The Crystalline *State*, London, G. Bell and Sons, Vol. II, 41.
MCGAVIN, S., 1962, *J. Mol. Biol.*, **5**, 275.
MOODIE, M. F., 1963, *Science*, **147**, 1173.
TOMLIN, S. G. and WORTHINGTON, C. R., 1956, *Proc. Roy. Soc. London, Series A*, **235**, 189.
WHITTAKER, E. J. W., 1955, *Acta Cryst.*, **8**, 261.
WORTHINGTON, C. R., 1960, *J. Mol. Biol.*, **2**, 327.