short communications

Diffraction by partially occupied helices

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An expression for cylindrically averaged intensity diffracted by a partially occupied helix (*i.e.* by a set of identical molecules bound to some, but not all, points of a discrete helix) is derived. The result is compared with previous studies and its application to muscle diffraction is discussed.

1. Introduction

Helical diffraction theory was developed in the fifties (Cochran *et al.*, 1952; Franklin & Klug, 1955; Klug *et al.*, 1958). Here we consider diffraction on a partially occupied helix (*i.e.* a set of identical groups of atoms that occupy some but not all points of a discrete helix). Structures of this kind arise from binding of identical large molecules to some but not all helix repeat units formed by smaller molecules. For example, in muscle, globular myosin heads bind to actin monomers on the thin filaments and partially occupy actin helix (Fig. 1*a*).

2. Structure, its transform and diffracted intensity

It is assumed that possible binding sites form a u_t helix (*i.e.* there are u helical points in t turns) with an axial distance d between the adjacent points. Some of the binding sites are occupied by identical molecules and, in the general case, the whole structure does not have any helix symmetry, although it has a period c, which is assumed to be a multiple of ud: $c = dU$, where $U = ru$, $T = rt$, and U, T and r are integers. In addition, $c = rc'$, where $c' = ud$ is the repeat distance of the underlying helix. The repeating binding pattern can be described by a set of U 'all or nothing' parameters, ρ_s:

$$\rho_s = \begin{cases} 1 & \text{if the } s\text{th binding site is occupied} \\ & \text{by a bound molecule,} \\ 0 & \text{if the } s\text{th site is free.} \end{cases} \quad (1)$$

As the structure is periodic, $\rho_s = \rho_{s+U}$. If K is the number of the occupied binding sites in a period c, then the average helix occupancy q is $q = K/U$ and $\sum_{s=0}^{U-1} \rho_s = \sum_{s=0}^{U-1} \rho_s^2 = K$.

Let the z axis of a cylindrical system of coordinates (r, φ, z) in real space coincide with the helix axis. If r_j, φ_j, z_j are the coordinates of the jth atom ($j = 1, 2, \ldots, M$) of the molecule bound to the zeroth binding site on the helix and f_j is the scattering factor of this atom, the coordinates of the same jth atom in the molecule bound to the sth site are r_j, $\varphi_j + 2\pi sT/U$ and $z_j + sd$, respectively. The transform of the whole structure is (Klug *et al.*, 1958; Vainstein, 1963)

$$F = \sum_l F_l(R, \Psi)\delta(Z - l/c),$$

$$F_l = \frac{1}{U} \sum_{s=0}^{U-1} \sum_{n=-\infty}^{n=\infty} \rho_s G_{nl}(R) \exp(in\Psi) \exp[2\pi is(l - nT)/U], \quad (2)$$

where

$$G_{nl}(R) = \sum_{j=1}^{M} f_j J_n(2\pi R r_j) \exp\{i[n(\pi/2 - \varphi_j) + 2\pi l z_j/c]\} \quad (3)$$

are the Fourier–Bessel structure factors; R, Ψ, Z are cylindrical coordinates in reciprocal space and J_n is the nth-order Bessel function of the first kind. Transform (2) is normalized for the total number U of available binding sites in a period c.

The cylindrically averaged intensity, $I_l(R)$, on the lth layer line for transform (2) can be calculated straightforwardly (Vainstein, 1963; Millane, 1991):

$$I_l(R) = \frac{1}{U^2} \sum_{n=-\infty}^{\infty} \sum_{s,k}^{U-1} \rho_s \rho_k |G_{nl}(R)|^2 \exp[2\pi i(s - k)(l - nT)/U]$$

$$= \sum_{n=-\infty}^{\infty} |G_{nl}(R)|^2 b_{l-nT}, \quad (4)$$

where

$$b_p = \frac{1}{U^2} \sum_{s,k=0}^{U-1} \rho_s \rho_k \exp[2\pi i(s - k)p/U]. \quad (5)$$

Expression (4) can be rewritten as

$$I_l(R) = \sum_n \sum_s |G_{nl}(R)|^2 b_s, \quad (6)$$

where n and s satisfy the selection rule

$$l = nT + mU + s \quad (7)$$

and m is any integer. This selection rule was obtained by Holmes *et al.* (1980). Expressions (4), or (6) and (7), provide a general formula for the cylindrically averaged intensity diffracted by a partially occupied helix. It contains only the Fourier–Bessel structure factors G_{nl} of a single repetitive group of atoms and the coefficients b_s, (5), which are determined by the binding distribution (1) and define the one-dimensional interference function of the binding sites,

$$L(Z) = \sum_s b_s \delta(Z - s/Ud). \quad (8)$$

$L(Z)$ is the transform of the auto-correlation function of the one-dimensional distribution function

$$A(z) = \frac{1}{U} \sum_s \rho_s \delta(z - sd). \quad (9)$$

In other terms, $L(Z)$ is the intensity diffracted by a linear array of K points with unit scattering amplitude which are distributed according to (9) and (1). The interference function $L(Z)$ is real; it has a period $1/d$ (*i.e.* $b_p = b_{p+U}$ for any p); $L(Z)$ is symmetric [*i.e.* $L(Z) = L(-Z)$, $b_p = b_{-p}$ for any p], and non-negative [$L(Z) \geq 0$, $b_p \geq 0$ for any p]. It

can be readily shown that $b_0 = q^2$ and the integral of $L(Z)$ over a period $1/d$ is equal to $q = K/U$ (*i.e.* $\sum_{s=0}^{U-1} b_s = q$).

3. Fully and partially occupied helices

For a fully occupied (*i.e.* a conventional) helix, $K = U$, $q = 1$, $b_0 = 1$ and $b_s = 0$ for all $s \neq mU$, where m is any integer. Hence the expressions (4), or (6) and (7), reduce in this case to the classical formula for the intensity, $I_l^f(R)$, diffracted by a discrete helix (Franklin & Klug, 1955; Klug *et al.*, 1958; Millane, 1991):

$$I_l^f(R) = \sum_{n \in \Im} |G_{nl}(R)|^2, \tag{10}$$

where the summation is over only those Fourier–Bessel terms that satisfy the helix selection rule: $n \in \Im$, $l = nT + mU$ (Cochran *et al.*, 1952). As $b_0 = q^2$, the expression (4) for the intensity diffracted by a partially occupied helix can be rewritten as

$$I_l = I_l^f q^2 + \sum_{n \notin \Im} |G_{nl}(R)|^2 b_{l-nT}, \tag{11}$$

where summation in the second term is over all those Fourier–Bessel terms G_{nl} that do not satisfy the helix selection rule, *i.e.* for all n and l for which $l \neq nT + mU$ for any integer m. The first term in (11) formulates the so-called square law of intensity. According to this law, the intensity diffracted by a partially occupied helix is the intensity diffracted by a fully occupied helix scaled with the square of its average occupancy. As can be seen from (11), the intensity diffracted by a partially occupied helix contains contributions from all Bessel functions, not only those satisfying the helix selection rule. As $b_s \geq 0$, the layer-line intensity diffracted by a partially occupied helix, $I_l(R)$, is higher than that predicted by the square law, $q^2 I_l^f(R)$.

A formula similar to (4), although with some misprints, was obtained by Gu & Yu (1999), who, however, did not notice that the 'all or nothing' occupation (1) induces a regular deviation from the square law of intensity.

4. Modulated helix

Instead of using the 'all or nothing' discrete binding parameters (1), Holmes *et al.* (1980) represented a partially occupied helix as a fully occupied helix modulated with a one-dimensional continuous binding probability function, $Q(z)$, $0 \leq Q(z) \leq 1$. They obtained the transform F^M of the modulated structure,

$$F_l^M = \sum_s \sum_n q_s G_{nl}(R) \exp(in\Psi), \tag{12}$$

where q_s are the coefficients of the Fourier series representing the function $Q(z)$. For $Q(z)$ having period c, integers n, l and s in (12) satisfy the selection rule (7). The cylindrically averaged intensity I_l^M that can be derived from transform (12) is

$$I_l^M(R) = \sum_n \sum_s |G_{nl}(R)|^2 |q_s|^2 \tag{13}$$

with the selection rule (7). Expression (13) is identical to (6) if $b_s = |q_s|^2$ for any s. This takes place if the binding probability function $Q(z)$ coincides with the discrete distribution function $A(z)$ defined by (9) and (1), *i.e.* if $Q(z)$ is essentially discrete. Otherwise, *i.e.* if the probability function $Q(z)$ has intermediate values between 0 and 1, (13) underestimates the intensity $I_l^M(R) < I_l(R)$ because in this case $\sum_{s=0}^{U-1} |q_s|^2 < \sum_{s=0}^{U-1} b_s = q$, where q is the average helix occupancy. For an extreme example of a randomly partially modulated helix (this applies only if r is large enough), the probability approach gives $q_s =$
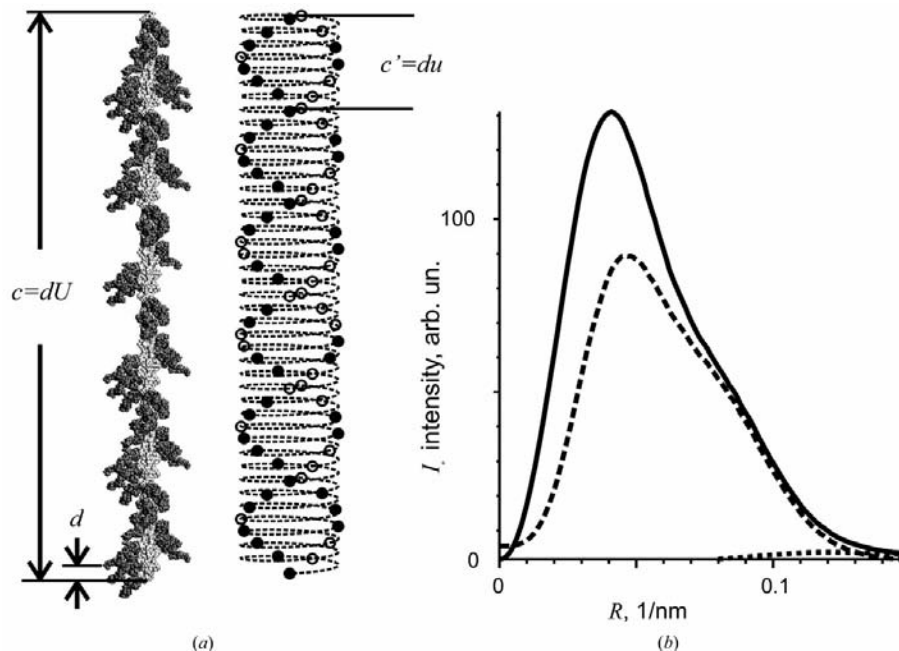

Figure 1
(*a*) An actin filament (grey) partially decorated by myosin heads (dark grey). High-resolution structures of actin, myosin head and their complex were taken from Rayment, Holden *et al.* (1993) and Rayment, Rypniewski *et al.* (1993). The actin binding pattern (closed circles) was obtained assuming that a myosin head binds the closest actin monomer on one of six surrounding actin filaments. The actin filament shown in (*a*) is decorated by the heads originating from three neighbouring myosin filaments. A myosin filament in skeletal muscle is assumed to be a three-strand 3_1 helix with a period of 42.9 nm. Actin is approximated by a 13_7 helix; $d = 2.75$ nm; $c = 214.5$ nm; $r = 6$, $U = 78$; $q = 45/78$. Except for the main term $b_0 = q^2 = 0.333$, the binding pattern has only one pronounced term of the interference function (8), $b_{30} = 0.080$. All other b_s are <0.005. (*b*) The cylindrically averaged intensity of the first actin layer line, I_6, calculated using expression (6) with the selection rule (7), $l = 42n + 78m + s$ (continuous line), or with only the first term of (11), *i.e.* the square law (dashed line); the intensity diffracted by actin itself is shown by the dotted line. The deviation from the square law for I_6 mainly arises from the $b_{-30}G_{-1,6}$ term of (6), proportional to J_{-1}.

0 for all q_s except $q_0 = q_{mU} = q$, where m is any integer. In this case, I_l^M is equal to the first term of (11), *i.e.* obeys the square law and neglects any contribution from the Bessel functions that do not satisfy the helix selection rule.

5. Application to muscle diffraction

An obvious application of the theory presented here is muscle diffraction where myosin heads bound to actin filaments partially decorate the actin helix and increase the intensities of the actin layer lines (Huxley & Brown, 1967). It follows from (11) that the square law that was used by Huxley & Kress (1985), Yagi (1996), Bershitsky *et al.* (1997), Gu & Yu (1999), Kraft *et al.* (1999), Tsaturyan *et al.* (1999) and Bordas *et al.* (1999) for the estimation of the number of myosin heads bound to actin during muscle contraction is incorrect. For example, for an actin helix that is periodically decorated by bound myosin heads as shown in Fig. 1(*a*), the difference between the first actin layer-line intensity calculated using expressions (6) and (7) and that predicted by the square law is quite substantial (Fig. 1*b*). The error in the estimation of the number of bound heads using the square law depends on the binding pattern [(1) and (9)] and can be as much as 70%.

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