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Labelling of Thin Filaments by Myosin Heads in Contracting and Rigor Vertebrate Skeletal Muscles

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Abstract

A method of crystallographic analysis to identify myosin heads interacting with specific actin sites in vertebrate striated muscles is described. It is based on a Fourier transform of a helix in which probability of occurrence of subunits varies periodically. It predicts the presence of layer-lines at 1/24 and 1/10.4 nm^{-1} which are experimentally observed in contracting and rigor vertebrate striated muscles, showing that the myosin heads are interacting with specific sites on actin but are still maintaining their average 14.5 nm axial periodicity.

1. Introduction

In a vertebrate striated muscle, the thick filament has a helix of myosin heads with an axial subunit repeat of *ca* 14.3 nm and a helical pitch of *ca* 42.9 nm in the relaxed state, while the thin filament has a helix of actin monomers with an axial subunit repeat of *ca* 2.73 nm and a helical pitch of approximately 36 nm (Squire, 1981). In contraction and rigor the axial repeat of the heads is *ca* 14.5 nm. Although these repeats in the two filaments are incommensurate, it has been suggested that the myosin heads in contracting muscle interact with actin maintaining their axial repeat. This suggestion is mainly based on the high intensity of the 14.5 nm meridional reflection in the X-ray diffraction pattern (Yagi, O'Brien & Matsubara, 1981; Huxley, Faruqi, Kress, Bordas & Koch, 1982; Bordas *et al.*, 1993) and in the optical diffraction pattern from electron micrographs (Tsukita & Yano, 1985; Hirose, Lenart, Murray, Franzini-Armstrong & Goldman, 1993).

However, there may be myosin heads that are in the vicinity of actin but not diffracting with actin-based periodicities. These will also contribute to the intensity. Therefore, it is not certain that the strong reflection at 1/14.5 nm^{-1} comes from the heads making a specific contact with actin. In fact, there has been no conclusive evidence that myosin heads are attached to a specific binding site on actin during contraction: although the actin layer-lines are enhanced, structural changes in the thin filament itself may account for at least part of the intensity changes (Wakabayashi, Ueno, Amemiya & Tanaka, 1988).

In a vertebrate striated muscle in rigor, where the myosin heads are making a specific contact with actin, the binding of myosin heads under the influence of the 14.5 nm repeat is supposed to cause a 'beat' in the diffraction pattern, creating layer-lines at orders of approximately 72 nm which is an approximate least common multiple of the 14.5 and 36 nm periodicities (Huxley & Brown, 1967; Haselgrove & Reedy, 1978; Squire & Harford, 1988). However, a crystallographic analysis of these layer-lines has not been made.

I propose a crzystallographic way to identify cross-bridges which are undergoing specific binding to actin under the influence of their 14.5 nm repeat.

2. Crystallographic formulation

2.1. *Insect flight muscle*

Holmes, Tregear & Barrington Leigh (1980) described in the case of an insect flight muscle in rigor a Fourier transform of a cross-bridge array in which all myosin heads are bound to a specific site on actin with the same conformation. In order to simulate the pattern of cross-bridges in the muscle (the so-called double chevron), they introduced a spatial modulation function $Q(z)$, which takes account of the unequal availability of binding sites along the actin helix. z is a cylindrical polar coordinate along the filament axis in real space. $Q(z)$ varies between 0 and 1. In the case of insect flight muscle, $Q(z)$ is a periodic function with a period of 38.5 nm, which can be expanded as,

$$Q(z) = \sum_{s=-\infty}^{\infty} q_s \exp[-i(2\pi s W z/c)], \qquad (1)$$

where c (= 77.0 nm) is the actin repeat, q_s the sth Fourier component of Q and W (= 2) the number of regions of head binding in an actin repeat. The Fourier transform of $Q(z)$ is,

$$T(Q) = q_0\delta(Z-0) + q_{+1}\delta(Z-1/38.5) + q_{-1}\delta(Z+1/38.5) + q_{+2}\delta(Z-2/38.5) + q_{-2}\delta(Z+2/38.5) + \ldots,$$

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where δ is the Dirac delta function, Z the cylindrical polar coordinate in reciprocal space corresponding to z, and $q_s = q^*_{-s}$. The transform of such an actin-labelling cross-bridge is,

$$F[R, \Psi, (l/c)] = \sum_n \sum_s q_s G_{nl}(R) \exp[in(\Psi + \pi/2)], \quad (2)$$

where $R, \Psi, l/c$ are the cylindrical polar coordinates in reciprocal space, l is given by $-13n + 28m + 2s$ as defined by the symmetry of the thin filament (*i.e.* 28 actin subunits in 13 turns of the genetic helix). G_{nl} is a Fourier transform of a myosin head which is given by,

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

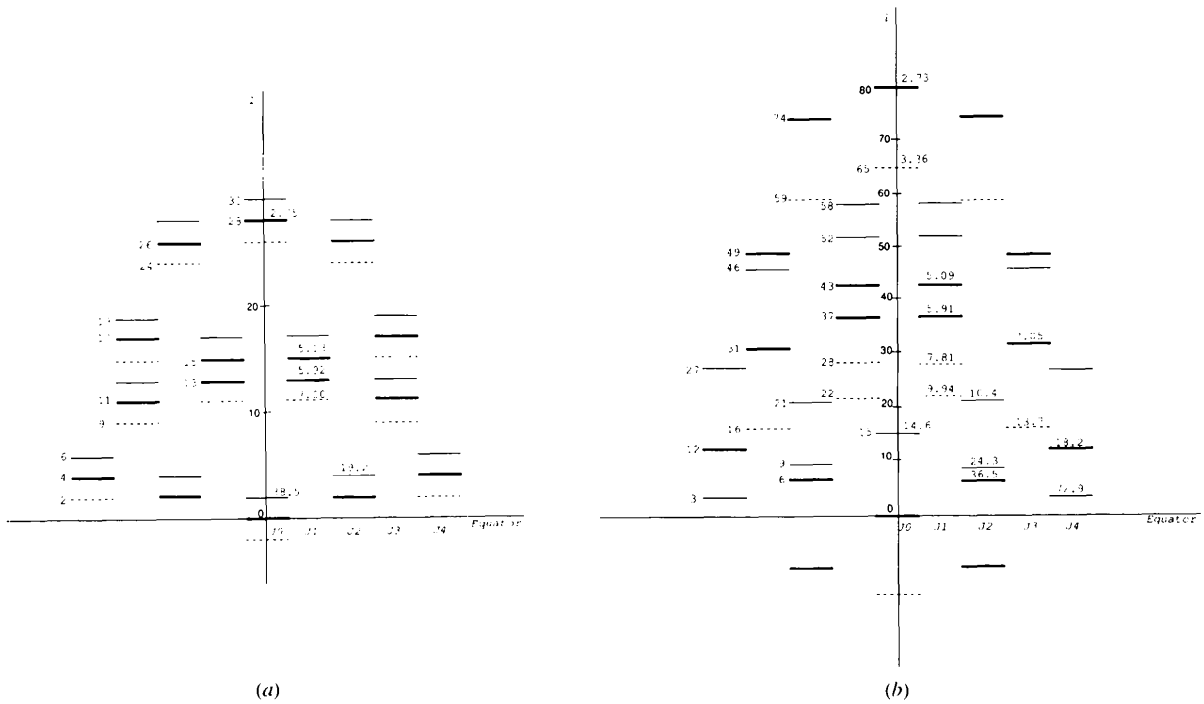
as in the usual formulation of the Fourier transform of a helix (Klug, Crick & Wyckoff, 1958) where N is the number of atoms in the myosin head, n the order of the Bessel function J, f_j the scattering factor of the atom j, and r_j, φ_j and z_j represent its cylindrical polar coordinate.

The effect of such a periodic modulation is to produce images of the cross-bridge transform shifted along the Z axis by multiples of $1/38.5\,\text{nm}^{-1}$ weighed by the corresponding value of q_s (Fig. 1*a*).

2.2. *Vertebrate striated muscle*

In the case of a vertebrate striated muscle in contraction and rigor, there is a prominent 14.5 nm meridional reflection. If at least part of the intensity of this reflection is a result of myosin heads labelling the actin filament, there is an induced perturbation with a period of either 14.5 nm or a multiple of it. Myosin heads cannot bind to actin maintaining their 14.5 nm repeat because the thin filament does not have such a repeat.

However, the explanation by Holmes *et al.* (1980) requires only an average repeat in the pattern of labelling of actin with myosin heads. Thus, the spatial modulation function (1) can be written as,



(*a*) (*b*)

Fig. 1. Schematic drawings of layer-lines from the myosin-labelled thin filaments of muscle. (*a*) For insect flight muscle in which myosin heads periodically bind to the thin filament with a 38.5 nm axial repeat. The selection rule is $l = -13n + 28m + 2s$ with an axial unit length of 77.0 nm. The solid bold lines are layer-lines expected from the thin filament randomly decorated with myosin heads. The periodic binding causes other layer-lines to appear. The thin layer-lines are due to shifting the solid layer-lines by $1/38.5\,\text{nm}^{-1}$ axially, while the dotted lines by $-1/38.5\,\text{nm}^{-1}$. Only major layer-lines are shown. Layer-lines are also expected to appear by shifting the solid layer-lines by $\pm n \times 1/38.5\,\text{nm}^{-1}$ ($n = 2, 3, \ldots$), but are not shown. The lateral positions of the layer-lines correspond to their principal Bessel orders indicated along the equator. The numbers over some of the layer-lines is the Bragg spacing (in nm), while the number in the left of a layer-line is its index (l). (*b*) For vertebrate striated muscle during contraction and rigor in which myosin heads periodically bind to the thin filament with a 14.58 nm axial repeat. The selection rule is $l = -37n + 80m + 15s$ with an axial unit length of 218.7 nm. It should be remembered that in reality the lateral position of the peak in a layer-line depends not only on its Bessel order but also on the radial coordinate of the molecule and its shape. In general, myosin heads are located at a higher radius than actin and so the layer-lines arising from them (the thin and broken layer-lines in the figure) will appear closer to the meridian.

$$Q(z) = \sum_{s=-\infty}^{\infty} q_s \exp[-i(2\pi sz/14.58)],$$

that is, $c = 14.58$ nm and $W = 1$. Its Fourier transform is,

$$T(Q) = q_0\delta(Z - 0) + q_{+1}\delta(Z - 1/14.58) + q_{-1}\delta(Z + 1/14.58) + q_{+2}\delta(Z - 2/14.58) + q_{-2}\delta(Z + 2/14.58) + \ldots.$$

The symmetry of the thin filament in this muscle can be approximated by 80 subunits of actin in 37 turns of the genetic helix within an axial repeat of 218.7 nm (Squire & Harford, 1988; Bordas *et al.*, 1993). This axial repeat is also 15×14.58 nm. The selection rule is $l = -37n + 80m + 15s$. The full Fourier transform of the cross-bridge array is given by (2) and (3), as is the case with an insect flight muscle.

It should be pointed out that the presence of an exact match between the thick and thin filament periodicities is not required for this interpretation. The 218.7 nm repeat is used as an approximation only for the purpose of indexing layer-lines. Therefore, the 14.58 nm periodicity is just an assumption, not a result of myosin–actin interaction.

Considering that higher orders of the 14.5 nm meridional reflection are much weaker, we will consider only the first three terms in $T(Q)$. The first term of $T(Q)$ gives the diffraction which is identical to the one from the thin filament itself, giving layer-lines from a 'decorated' thin filament (Holmes *et al.*, 1989; Amos, Huxley, Holmes, Goody & Taylor, 1982). The second and third terms of $T(Q)$ give rise to sets of layer-lines which are shifted axially by $\pm 1/14.58\,\text{nm}^{-1}$ (Fig. 1*b*). The two layer-lines at 1/10.4 and $1/24.3\,\text{nm}^{-1}$ appear as a result of shifting the layer-lines at $1/36.5\,\text{nm}^{-1}$ on each side of the equator by $1/14.58\,\text{nm}^{-1}$. These layer-lines have an intensity distribution of J_2. Other layer-lines are expected at 1/13.7, 1/9.94, $1/7.81\,\text{nm}^{-1}$. These are created by shifting the layer-lines at 1/7.0, 1/5.9, $1/5.1\,\text{nm}^{-1}$ by $-1/14.58\,\text{nm}^{-1}$. In the medium-angle region, a meridional reflection is expected at $1/3.36\,\text{nm}^{-1}$, which is created by shifting the meridional reflection at $1/2.73\,\text{nm}^{-1}$ by $-1/14.58\,\text{nm}^{-1}$.

Bordas *et al.* (1993) proposed that myosin heads periodically label 16-actin units whose periodicity is $2.734 \times 16 = 43.74$ nm. This would correspond to a modulation of 3×14.58 nm. It should be noted that this reduces to the above when one neglects the weak terms.

2.3. *Difference between insect and vertebrate muscles*

The difference in the labelling patterns of an insect flight muscle and a vertebrate striated muscle results from the fact that the periodicities in the two filaments are commensurate in the former while they are not in the latter. Hence, in the case of an insect muscle the new layer-lines all overlap the original ones while they appear as new layer-lines with different axial spacings in a vertebrate muscle (Figs. 1*a* and 1*b*).

A vertebrate muscle during contraction is also different from an insect muscle in rigor in that the conformation of the head (that is, the crystallographic form factor) may differ considerably from one to another. For example, part of the head which is close to the tail may take different conformations depending on its chemical or tension developing state. This part of the myosin head does not follow the symmetry of the actin helix. However, it can probably be assumed that the part of myosin head which is making contact with actin is fixed at certain amino-acid residues on actin and hence follows the actin helix. Only this part of the head contributes to the new layer-lines described above. The heads which are not distributed with the symmetry of the thin filament do not contribute to these new layer-lines, even if they may be interacting with actin. The detached heads may be axially aligned with a 14.5 nm interval and hence contribute to the 14.5 nm meridional reflection, but again not to the new layer-lines.

It is interesting to note that, in crustacean striated muscles in rigor, strong layer-lines are observed at 1/38 and $1/19\,\text{nm}^{-1}$ but not at $1/24\,\text{nm}^{-1}$ (Wray, Vibert & Cohen, 1978; Maeda, Matsubara & Yagi, 1979; Namba, Wakabayashi & Mitsui, 1980), showing that the binding of myosin heads is not influenced by the 14.5 nm axial repeat in the thick filament. Since the helical parameters of the thick filament do not match those of the thin filament in these muscles, the periodic 38 nm attachment does not seem to be because of a regular three-dimensional arrangement of the two filaments as in an insect flight muscle: possibly, the presence of troponin may affect the binding (Wray *et al.*, 1978).

3. Experimental

Among the predicted layer-lines, the following layer-lines are actually observed in vertebrate striated muscles.

$1/24.3\,\text{nm}^{-1}$ ($l = 9$): during contraction (Bordas *et al.*, 1993) and in rigor (Huxley & Brown, 1967; Haselgrove, 1975; Squire & Harford, 1988; Yagi, 1992). Also in an optical diffraction pattern from electron micrographs of rigor muscles (Craig, Alamo & Padron, 1992; Hirose *et al.*, 1993).

$1/10.4\,\text{nm}^{-1}$ ($l = 20$): during contraction (Bordas *et al.*, 1993) and in rigor (Wakabayashi *et al.*, 1988).

$1/9.94\,\text{nm}^{-1}$ ($l = 22$): in rigor (Yagi, unpublished observation on the diffraction pattern taken from a frog striated muscle treated with *N*-ethylmaleimide).

$1/3.36\,\text{nm}^{-1}$ ($l = 65$): during contraction (Bordas, personal communication on the diffraction pattern from an isometrically contracting frog muscle).

The explanation described above predicts the axial separation between the 24 and 14.5 nm layer-lines to be identical to that between the equator and the 36 nm layer-line. Experimentally, the ratio of these axial spacings varies between 0.992 and 1.017 (Huxley & Brown, 1967; Haselgrove, 1975; Squire & Harford, 1988; Craig *et al.*, 1992; Bordas *et al.*, 1993). Since an accurate measurement of the spacings of the 36 and 24 nm layer-lines is difficult because of the diffuse nature of the layer-lines, the values cited in these literatures may be consistent with the explanation presented above. In the best ordered rigor pattern (Yagi, 1992), the reported value was 1.030, but a new measurement on many original diffraction patterns gives a value close to unity. A more accurate measurement of these spacings would be useful to confirm that the myosin heads are indeed diffracting as predicted.

In a rigor pattern taken from a frog muscle treated by *N*-ethylmaleimide (Yagi, 1992), the 24.3 nm layer-line has the strongest sampling at the (1,1) row line. In other less sampled rigor patterns, the layer-line has a broad peak in the similar region. Since the layer-line has a J_2 contribution, this indicates the center of mass of the bound head is at about 11 nm from the thin filament axis. This is slightly larger than the previously reported values (Amos *et al.*, 1982; Milligan & Flicker, 1987). Although the variation in the structure factor of the head may shift the position of the peak, the large value suggests that part of the myosin head near the tail may be well ordered in a rigor muscle.

Bordas *et al.* (1993) reported that the intensity of the 24 nm layer-line at plateau of tension was 70–80% of that in rigor, while the intensity of the 37 nm layer-line was only 15–20% of that in rigor. The weak 37 nm layer-line suggests that the number of heads bound to actin is small. However, the 14.5 nm meridional reflection is much stronger than in rigor, suggesting that the axial arrangement of heads is more regular during contraction. This may account for the high intensity of the 24 nm layer-line.

The time course of the intensity change of the layer-line at $1/10.4\,\mathrm{nm}^{-1}$ during development of tension has been measured by Bordas *et al.* (1993). After the restimulation from 30% remnant tension, the increase in intensity parallels the development of tension, suggesting that the number of heads attached to actin following the symmetry of the actin helix increases as the tension rises.

4. Conclusions

The analysis presented here shows that the intensities of the layer-lines at 1/24.3 and $1/10.4\,\mathrm{nm}^{-1}$ are important in studying the binding of myosin heads to actin. The intensities of these layer-lines are not dependent on the structure of actin: they solely depend on the number and conformation of myosin heads which are attached to a specific binding site on actin but still maintaining their own axial periodicity. Therefore, these layer-lines are most informative of the structure of the myosin heads which are actively interacting with actin.

It is not surprising that the myosin heads maintain their axial repeat during contraction. Since the heavy meromyosin portion of myosin originates from the thick filament backbone with a 14.5 nm repeat, the mean positions of the heads have to be separated with 14.5 nm intervals. This periodicity becomes more enhanced if the bound heads can take only restricted conformation. Also, since the head and S2 portion are under tension, they may not have much freedom of movement.

The observation that these layer-lines appear during contraction (Bordas *et al.*, 1993) shows that myosin heads are attached to actin at a fixed binding site. It is also concluded that the binding of heads to actin in rigor is influenced by the 14.4 nm periodicity in the thick filament.

This crystallographic approach described here complements modelling studies (Squire & Harford, 1988): modelling enables one to investigate detailed structural aspects while the present approach is more convenient to analyse an experimentally observed diffraction diagram without referring to a particular model. Both approaches should be employed to interpret fully diffraction patterns from muscles.

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