MODELING RIGOR CROSS-BRIDGE PATTERNS IN MUSCLE

I. Initial Studies of the Rigor Lattice of Insect Flight Muscle

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ABSTRACT We have undertaken some computer modeling studies of the cross-bridge lattice observed by Reedy in insect flight muscle so that we can investigate the geometric parameters that influence the attachment patterns of cross-bridges to actin filaments. We find that the appearance of double chevrons along an actin filament indicates that the cross-bridges are able to reach 10–14 nm axially, and about 90° around the actin filament. Between three and five actin monomers are therefore available along each turn of one strand of actin helix for labeling by cross-bridges from an adjacent myosin filament. Reedy's flared *X* of four bridges, which appears rotated 60° at successive levels on the thick filament, depends on the orientation of the actin filaments in the whole lattice as well as on the range of movement in each cross-bridge. Fairly accurate chevrons and flared *X* groupings can be modeled with a six-stranded myosin surface lattice. The 116-nm long repeat appears in our models as "beating" of the 14.5-nm myosin repeat and the 38.5-nm actin period. Fourier transforms of the labeled actin filaments indicate that the cross-bridges attach to each actin filament on average of 14.5 nm apart. The transform is sensitive to changes in the ease with which the cross-bridge can be distorted in different directions.

INTRODUCTION

The active force for muscle contraction is thought to be generated by the heads of the myosin molecules projecting from the thick filaments (Huxley, 1969). These heads (called cross-bridges) attach to actin monomers in the adjacent actin filaments and change their configuration, thus pulling the actin and myosin filaments past each other, so it is important to investigate the motions of the cross-bridges, and discover what geometric factors influence the ability of the cross-bridges to attach to actin.

Unfortunately, it is not yet possible to freeze an actively contracting muscle for studying its cross-bridges in the electron microscope, but when a muscle passes into rigor, cross-bridges attach to actin to form an extensive, stable three-dimensional lattice of linkages between actin and myosin filaments. It is assumed that only those

cross-bridges that attach to actin are preserved by the fixation-embedding process (Reedy, 1967, 1968; Squire, 1972), and Reedy has described in detail the arrangement of these attached cross-bridges in the lattice of rigor insect flight muscle. The selection of bridges that become attached will depend not only on the structure of the thick and thin filaments (which determine the origins of the actin and myosin monomers in the relaxed muscle) but also on the ability of the myosin molecules to move in the interfilament space to bind to actin.

We have undertaken a series of model building studies using a computer. We started with a six-stranded myosin filament (Squire, 1972; Tregear and Squire, 1973), and investigated quantitatively how far along and around the actin filament the cross-bridge might be permitted to search for an actin-binding site. We describe here our initial studies of insect flight muscle, enlarging on Haselgrove's (1978) preliminary report, although we have extended our studies to include the structure of vertebrate skeletal muscle that we will describe in a later paper. We use both a real space comparison of our models with Reedy's micrographs, and reciprocal space comparison of the Fourier transform of our models with light diffraction patterns from Reedy's work (1967, 1968, and manuscript in preparation; see also Beinbrech, 1977).

METHODS

Computations

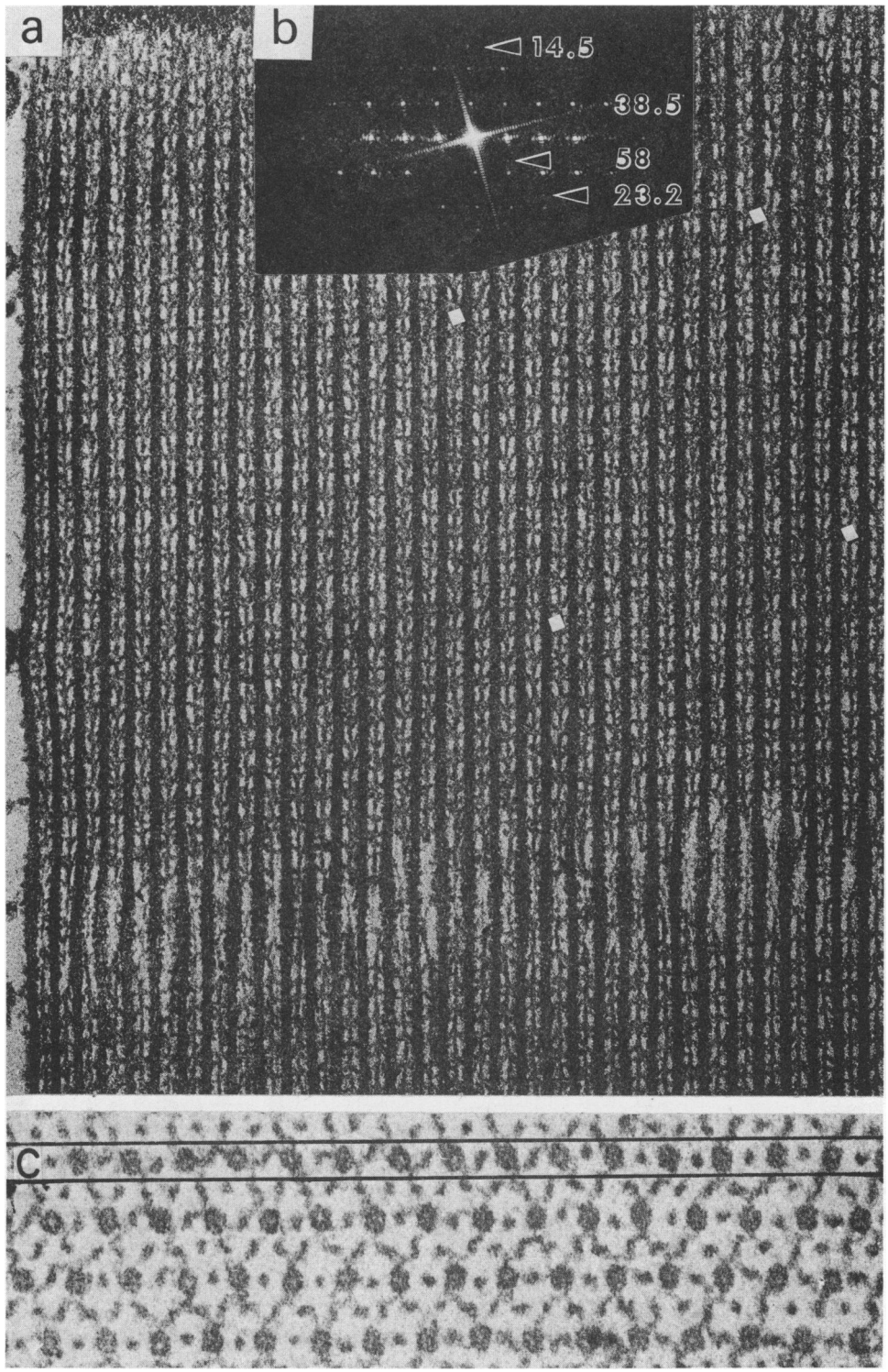
The calculations were performed on the PDP10 computer of the Medical School of the University of Pennsylvania.

Description of the Model

The calculations performed here were made to simulate the structure of the rigor insect flight muscle (Fig. 1). A six-stranded myosin filament sits at the lattice point of a hexagonal lattice surrounded by six actin filaments, and the actin filaments sit at the dyad positions flanked by just two myosin filaments. Because the structure of the filament lattice is so highly crystalline, the interactions made between each filament and the surrounding ones will be very similar (if not identical) across the lattice, so we computed only the interactions between one myosin filament and the surrounding actins, or one actin and the surrounding myosins.

It is convenient when describing helical filaments to use a system of cylindrical polar coordinates in which the axis of the filament is the cylindrical axis. The position of any point may then be described in terms of (*a*) an axial distance from an arbitrary reference line drawn perpendicular to the axis, (*b*) an azimuthal angle measuring the angular position around the filament from the arbitrary reference line, and (*c*) a radial distance from the axis.

FIGURE 1 Actual images of rigor cross-bridge features to be modeled for *Lethocerus* flight muscle. (*a*) Axial 38.5-nm repeat of cross-bridge chevrons, most of them doubled, appears in myac-type single filament layer; parallel horizontal lines in *c* show orientation and thickness of myac layer (Reedy, 1967). (*b*) Optical transform from indicated area (white squares) of micrograph exhibits six of the first nine orders of 116-nm long repeat; four appear on meridian in this example. (*c*) Thinnest (18 nm) transverse sections show flared *X* pattern of four rigor bridges per filament at the level of a single crown. Flared *X*'s rotate counter-clockwise in 60° steps as section level moves deeper by 14.5 nm steps, from lower right to upper left of *c*. Magnifications: (*a*) ×95,000; (*c*) ×210,000.


a
b
14.5
38.5
58
23.2
c

The positions of the cross-bridges on the surface of the myosin filaments were represented by a two-dimensional array of numbers. The vertical columns represent the strands of cross-bridges, and the horizontal rows represent the periodic axial levels at which cross-bridges occur. The helical structure of one strand was defined in terms of the axial repeat of the cross-bridges (e.g., 14.5 nm), and the number of subunits, u, in t turns of the helix. Then, when the position of the first subunit (cross-bridge) on the first strand had been defined, the coordinates of all other bridges could be calculated from the position of each in the array.

Each actin filament was described as a single line of frames representing the genetic (single-stranded, tightly coiled, 5.1-nm pitch) helix. The helix had approximately 28 subunits in 15 turns, which generates the commonly recognized double-stranded right-handed helix which repeats every 38.5 nm (Hanson and Lowy, 1963; Depue and Rice, 1965).

The polar coordinates of the cross-bridges on the myosin filament and the directions of the adjacent actin filaments around the myosin filament were measured from an arbitrary reference line drawn normal to the myosin filament axis; the coordinate system describing each actin filament was then measured from a line joining the actin to the myosin filament at the axial level of the original reference line (Fig. 2). Each filament could be effectively rotated or moved axially by changing the coordinates of the first subunit with respect to the appropriate reference line. The radial positions of the myosin cross-bridges and the actin active-sites were unrestricted, partly because we have little knowledge of their values, but mainly because it seems likely to us that the radius is relatively unimportant in the interactions.

The position of each cross-bridge in turn was calculated, and its azimuthal coordinate was compared with the azimuthal position of each of the actin filaments: if the azimuthal position of the cross-bridge was within a defined angle Φ_M of the direction of an actin filament, then we assumed that the cross-bridge could reach that filament (Fig. 2). A search was then made along the appropriate actin filaments for any actin monomers that lay within an axial range $\pm R$ and within a defined azimuth $\pm\Phi_A$ of the direction of the myosin filament (see Fig. 2). Any actin monomer already labeled with a cross-bridge was ignored, and if more than one actin monomer was in range, then the one to be labeled by the cross-bridge in question was chosen on the basis of a weighting system. If the cross-bridge could reach no actin monomers within the defined range, then it was given a value of zero, but if it could attach somewhere, then both it and the labeled actin monomer were given a value of 1. Unlabeled actin monomers had the value zero. The initial weighting was chosen so that the most favorable attachment position was with no axial or azimuthal displacement around the actin as follows: if an actin monomer was within range but required an axial movement r and an azimuthal movement ϕ, the actin was assigned a probability of $\frac{1}{2}-\frac{1}{4}(|r|/R + |\phi|/\Phi_A)$. Attachment was made to the actin monomer with the largest probability

Display

When all the cross-bridges had been interrogated in turn, the computer had a record of which cross-bridges had attached, and which actin monomers were labeled by those cross-bridges. We chose two display presentations for attached cross-bridges to simulate two different views of the structure described by Reedy. One display presents the axial positions of attached cross-bridges, simulating an actin filament as seen when looking at the plane containing the actin filament and the two adjacent myosin filaments (see Fig. 1) although we do not include either of the two flanking myosin filaments in our display. We represent each cross-bridge by a line uniformly angled at 45°. The second display represents the six actin filaments around each myosin filament and shows the bridges attached at each level. Such a radial projection of the attached bridges is shown in Fig. 6; by imagining this lattice to be wrapped round a myosin filament, we can see which actin filaments are labeled with a cross-bridge at any level. Essentially the same display convention was used by Squire (1972).

Fourier Transforms

The array of cross-bridges attached to any actin filament was subjected to a standard fast Fourier transform routine: the positions of the numbers 1 represent the attached cross-bridges. To increase the resolution of the transform, the linear array of numbers was extended with a line of zeros beyond the originally calculated 300 subunit frames, to 1,024 frames.

RESULTS

Data Being Analyzed

The important features of rigor insect muscle structure which Reedy described and which we are attempting to interpret are:

(*a*) The cross-bridges attach to actin in pairs every 38.5 nm to give a double chevron appearance. Double chevrons are much more common than single ones, and the successive bridges within double chevrons usually appear not to be on successive actin monomers.

(*b*) The cross-bridges originate on the myosin filament only at distinct axial levels 14.5 nm apart along the filament. Reedy et al. (1973) call these levels "crowns," and we do so, too. In thin transverse sections, four cross-bridges from each crown can be seen to attach to four of the six adjacent actin filaments (Fig. 1 *c*). The two unlabeled actin filaments are usually on opposite sides of the myosin filament, so the attached cross-bridges form an "X" shape which Reedy (1967) refers to as a "flared X" because of the details of its shape. As one moves from one crown to the next along the myosin filament the orientation of the flared X rotates by 60° along a left-handed screw.

(*c*) There is evidence that the whole structure repeats axially every 116.0 nm.

(*d*) The optical diffraction pattern of a longitudinal section shows diffraction spots at the following orders of 116.0 nm: 1,2,3, 5,6, 8,9.

Initial Choice of Model Parameters

We have chosen to conduct our initial analysis to investigate the position where cross-bridges attach on the actin filaments rather than the angles which the individual attached bridges might make. The positions of the attachment probably depend on the following parameters:

(*a*) The number of heads on each cross-bridge that can attach independently of one another.

(*b*) The position of the cross-bridge origin on the myosin filament.

(*c*) The structure of the actin filament.

(*d*) The extent to which the cross-bridges can move around the myosin filament and around and along the actin filaments to find an acceptable actin monomer for attachment.

There are too many parameters here for useful modeling, so we reduced their number as follows for the initial analyses.

(*a*) We assumed that each cross-bridge has only one head.

(*b*) If the myosin filament is six-stranded (Squire, 1972; Reedy et al., 1973; Bullard

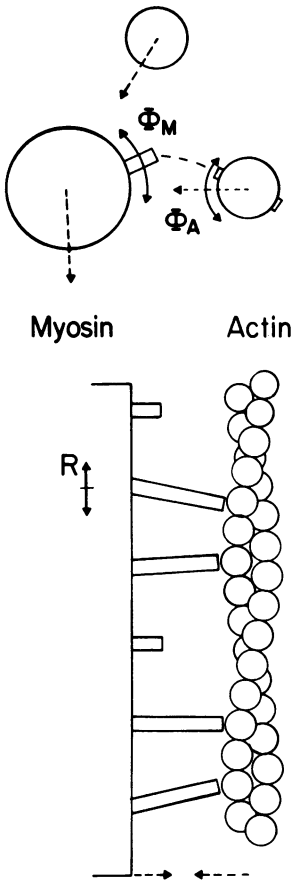


FIGURE 2

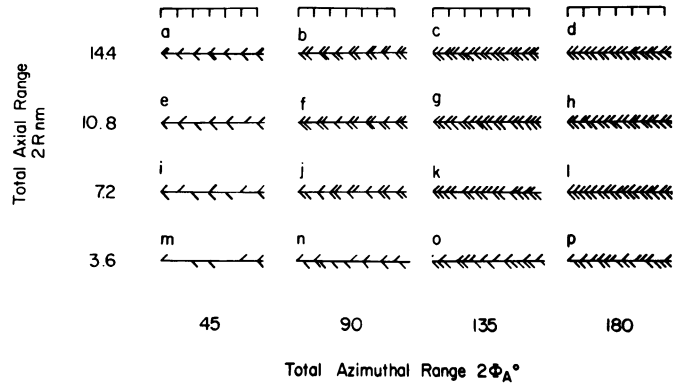


FIGURE 3

FIGURE 2 Diagram of adjacent actin and myosin filaments. The dashed arrows represent the reference lines used for the cylindrical polar coordinate systems describing each filament.

FIGURE 3 The appearance of the actin filament labeled with cross-bridges for a variety of pairs of parameters R and Φ_A. All cross-bridges are represented with exactly 45° tilt. The values for the total range ($2R$) and total azimuthal search round actin ($2\Phi_A$) are shown on the left of each line and the bottom of each column, respectively. Scale of 38.5-nm period is shown above each column.

and Reedy, 1973; Tregear and Squire, 1973), then at each cross-bridge level the bridges are spaced at 60° intervals around the myosin filament, so each bridge need move at most 30° to find an actin filament. 30° seems a reasonable azimuthal movement to permit for the cross-bridge so we simplified the calculations by keeping Φ_M at 30°, to allow just one cross-bridge to reach each actin filament at each cross-bridge level. In this model the helical structure of the myosin is obscured by the ability of the cross-bridges to move round the myosin, so we call it a nonhelical myosin. In such a myosin, cross-bridges appear as a series of six linear axial arrays along the filament surface, so for initial modeling we positioned one array of origins exactly opposite each

actin filament. With such a nonhelical model for the myosin filament, any value of $\Phi_M < 60°$ will give the same results because one cross-bridge is always able to reach only the facing actin filament. But if Φ_M is made to be 60° or more, then each cross-bridge could reach three actin filaments (the one facing it and one on either side), and the situation becomes complicated by the competition of two or more cross-bridges for two or more actin filaments. We therefore restricted ourselves to a value of $\Phi_M = 30°$.

(*a*) We initially described the actin and myosin filaments using the parameters measured by Miller and Tregear (1972), viz., myosin cross-bridge period = 14.5 nm, actin subunit period = 2.75 nm, actin helix half-pitch = 38.5. However, with very slight changes in the parameters we could create a structure that repeated exactly every 32 cross-bridge repeats and 6 actin half-periods. We also modeled with these "forced" parameters of 14.49 nm for myosin and 2.76 nm and 38.64 nm for the actin periods, and we drew identical conclusions with the two models.

Appearance of the Double Chevrons

When the simplifying assumptions above have been made, we can see that the attachment of cross-bridges to actin depends on the ability of the cross-bridges to move along and around the actin filament in search of a binding site. The movement may be described in terms of the axial range R and the azimuthal range Φ_A. Fig. 3 shows the appearance of the actin filament decorated by cross-bridges according to a variety of different sets of search parameters. In general, few actin monomers are in precisely optimal positions, so that bridges do have to search for actin monomers to attach to. If the axial range and azimuthal angle round actin over which the cross-bridges can

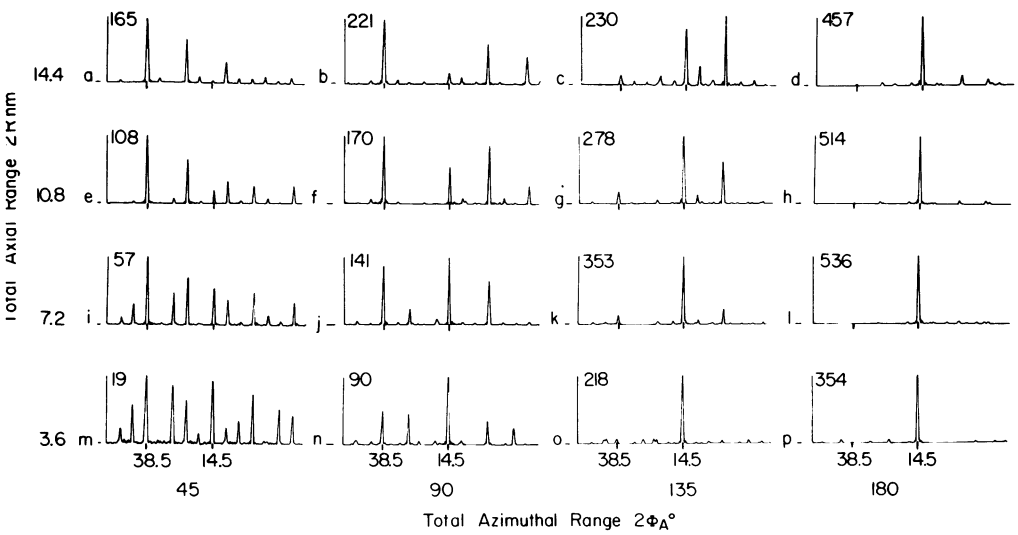
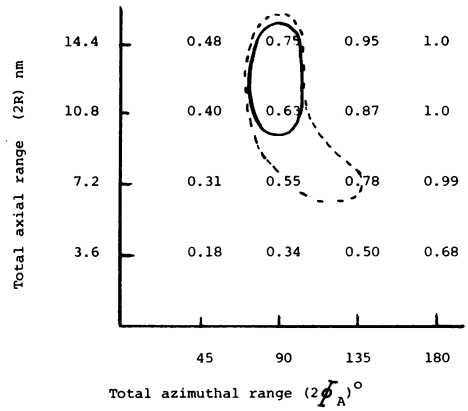


FIGURE 4 The intensity of the one-dimensional Fourier transform generated from each of the actin filament models shown in Fig. 3. Bridges were perpendicular, not 45°, for transforms. The positions of 38.5- and 14.5-nm reflections are indicated.

TABLE I

FRACTION OF TOTAL CROSS-BRIDGES THAT CAN ATTACH WITH DIFFERENT VALUES OF AXIAL AND AZIMUTHAL RANGE



The area surrounded by the solid line indicates the region of models with good double chevrons and flared *X*'s, whereas the dotted line indicates the region with less good but possible models.

search are small (as in the lower left-hand corner of the array), then very few will attach, whereas with a sufficiently generous axial and azimuthal range all the bridges are able to attach. Fig. 3 *b* and *f* show that with appropriate choice of range and azimuth, models can be produced in which the cross-bridges attach to simulate the double chevron grouping described by Reedy (1968). Table I shows the average fraction of cross-bridges which attach at each crown along the myosin filament for different values of 2 *R* and $2\Phi_A$. The solid outline shows the region for optimum modeling of double chevrons; the dotted outline shows the region where we still see double chevrons, including many where successive bridges attach to adjacent actin subunits (a feature rarely recognized in electron micrographs). Therefore we can see from Fig. 3 and Table I that for the nonhelical myosin model to produce double chevrons, the cross-bridges must be free enough to search over a total axial range ($2R$) of 10–14 nm, and to search for actin monomers within an azimuth ($2\Phi_A$) of about 90°.

Flared X

The above analysis shows that with a suitable choice of parameters for search movements by cross-bridges, two out of every three bridges will attach to actin filaments. With the actin filaments aligned as described by Reedy (Fig. 5 *b*) then at any level the cross-bridges can attach to four of the six surrounding actin filaments, and the sense of rotation of the *X* as we move along the myosin filaments is opposite to that of the screw sense of the two-stranded actin filament. The actin helix has a repeat of 38.5 nm, equivalent to 2.4 cross-bridge repeats, so the cross-bridges on level three or

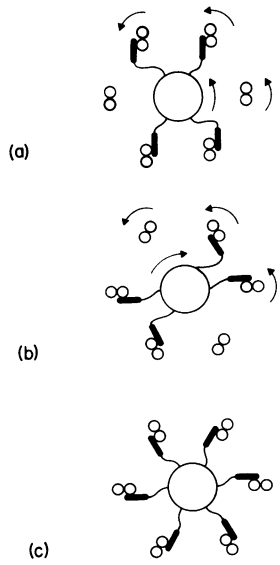


FIGURE 5

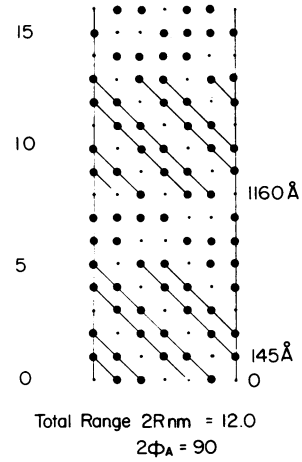


FIGURE 6

FIGURE 5 Diagrams of the three different crystallographically possible orientations of the actin filaments around the myosin filament, shown at an arbitrary level. The arrows indicate the way in which the actin filament structure and (in *a* and *b*) the observed flared *X* of attached cross-bridges both rotate as the filaments extend toward the reader. (*a*) All the actin filaments lie parallel to one another. (*b*) The filaments lie at 120° to each other but do not all point to the myosin filament at any one level (the favored model). (*c*) The filaments lie at 120° to each other but all point to the myosin filament at one level every 38.5 nm.

FIGURE 6 Diagram showing the levels (of 14.5 nm) at which cross-bridges attach to adjacent actin filaments (situated as in Fig. 5 *b*). The large circles represent attached cross-bridges. The left-handed helix generated by the attached bridges can be traced round the filament.

four do not have the same view of the actin filaments as the cross-bridges on level 1, and only after about 116 nm (eight cross-bridge repeats) do the cross-bridges again have a similar view of the actin filaments and again attach in similar positions. Fig. 6 shows a lattice in which the vertical lines each represent one of the six actin filaments surrounding the myosin filament (the left- and right-hand lines represent the same placement after circumscribing the myosin filament once), and the horizontal rows represent the levels of the "crowns" of cross-bridges at intervals of 14.5 nm along the myosin filament. At each crown the actin filaments labeled by a cross-bridge are marked by a large dot, and it can be seen from Fig. 5 that at the levels of most crowns (= any horizontal row) four of the six actin filaments are labeled. The thin diagonal lines trace out the way in which the four arms of the flared *X* rotate as one proceeds from crown to crown along the filament, and it can be seen that the structure is approximately the same every 116 nm. The *X* configuration (four bridges as two opposed pairs) is produced in these displays only when we use the values of R and Φ_A from the same region of the Table I matrix which successfully models the double chevrons.

We were interested to investigate the effect of other possible orientations of the actin

filament within the lattice. One possible situation occurs if the actin filaments all start together and are all oriented so that they face one myosin filament at one level (Fig. 5 *c*). Then wherever one actin can accept a cross-bridge, all six can do so at the same level; wherever one actin cannot accept a bridge, then none will do so at that level. The other possible configuration that retains some crystallographic sense arises if all the actins at any one level face in the same direction in the lattice (Fig. 5 *a*). In this case the attached cross-bridges again generate a rotating flared X but the sense of rotation on the myosin here is right-handed, the same as that of the actin double helix. Both the all-facing-myosin (Fig. 5 *c*) and the all-facing-one-way patterns (Fig. 5 *a*) of actin orientation thus fail to simulate the actual behavior of the flared X configuration observed in Reedy's micrographs.

The Long Repeat of 2 × 116 nm

For one of our models we intentionally forced the parameters of the actin and myosin helices to repeat exactly in phase after 231.8 nm. Thus every 232 nm the sequence of positions at which bridges attach to actin begins again, but because the actin filament has a pseudo-twofold rotation axis, the pattern of attached bridges is similar, although not identical after 232/2 = 116 nm. However, when we accepted the slightly different helix parameters from the X-ray work of Miller and Tregear (1972), we found as expected that the pattern of attachment of cross-bridges does not repeat *exactly* after 232 nm but that the pattern still has features that repeat approximately after 116.0 nm. Thus the long repeat seems to be a feature common to any model in which the actin and myosin periods are nearly (or exactly) in phase again after 231.8 nm. We are in fact looking at the "beat" between the two periods.

Fourier Transforms

To distinguish between the various models that look similar by eye, we calculated the axial Fourier transform of the structures. (We represented the cross-bridge as a perpendicular rod, and thus ignore at this stage the effect that the shape or axial tilt of the cross-bridge will have.) We compared our transforms with the optical diffraction patterns given by Reedy's micrographs, and assumed that the backbone of the filaments contribute very little to the transform.

Fig. 4 shows the intensity of the transforms obtained from each of the models shown in Fig. 3. The predominant reflections we find are the same as seen from Reedy's micrographs: namely, the 1,2,3, 5,6, 8,9 orders of the long repeat of 116 nm, although we do not in general see all of these reflections in any one transform, except when few bridges attach (lower left-hand corner of Fig. 4). Reedy does not record the 4th or 7th orders of the 116 nm repeat, and these reflections are absent from our calculations except for models with very few attached bridges: Fig. 4 *m* shows a weak 7th order, and a similar model using the "forced" actin and myosin parameters (not shown here) displays the 4th order, too. By varying the appropriate pitch values of the actin and myosin helices, and watching the shapes in the transform, we were able to determine which features of the model are responsible for the different reflections in the diffrac-

tion pattern and why the 4th and 7th orders of 116.9 nm do not appear. The predominant reflections are those at 38.5 and 14.5 nm. The 38.5-nm reflection comes from the spacing of the double chevron which is determined solely by the pitch of the actin helix. The 14.5-nm reflection changes its spacing when the myosin cross-bridge repeat changes, but because we are transforming an array of actin monomers it must indicate the mean spacing between successive cross-bridges along the actin filament (although the actin sites are every 27.5 nm apart).

The other reflections all appear as a result of beats between these two basic frequencies, and the origin of the reflections that we generate on our transforms are shown in Table II. Thus we were able to generate meridional reflections corresponding to the major spacings found in Reedy's patterns, although we were not able with our simple one-dimensional model to produce all of these in one pattern.

The intensities of the different reflections depend on the values of the axial and azimuthal ranges R and Φ_A. For small azimuthal ranges (two left-hand columns in Fig. 6), the axial positions of cross-bridge attachments are determined predominantly by where the actin subunits occur near the optimum azimuth for attachment, and the 38.5-nm repeat of the actin helix dominates the pattern, but when the axial range of cross-bridge is restricted (lower two lines in Fig. 6), then the cross-bridge can only attach regularly at 14.5-nm intervals, and this myosin repeat is then strong. When both the axial and azimuthal ranges are large (as in the upper, right-hand corner of Fig. 6),

TABLE II

SOURCES OF THE REFLECTIONS SEEN IN OUR MODELING AS ORDERS OF 116 NM

(*a*) Order of 116	(*b*) Nominal spacing	(*c*) Source
	nm	
1	116	Third order, 38.5 – first order, 14.5
2	58	14.5 – second order 38.5
3	38.5	38.5
4	29	Not seen
5	23.2	14.5–38.5
6	19.3	Second order, 38.5
7	16.5	Not seen
8	14.5	14.5
9	12.8	Third order, 38.5
10	11.1	Not seen
11	10.6	14.5 + 38.5

The reflections are probably "beats" from two basic periodicities, one at 38.5 nm (from actin), the other at 14.5 nm (from myosin). (Columns a and b) Spacing of reflection and indexing on 116-nm period. (Column c) Indication of how the reflection is produced by the difference or sum of the spacing of two other reflections, one of which will be an order of 38.5 nm, the other an order of 14.5 nm. The spacings are quoted as real space values, although additions are performed in reciprocal space, e.g., $1/116 \simeq 3/38.5 - 1/14.5$.

there is usually more than one actin attachment site available for each cross-bridge. The choice of site is then made on the basis of the "weighting" function, which for these models is designed to keep the cross-bridges close to their points of origin on the myosin filament, so the myosin repeat period of 14.5 nm is dominant in the transform.

Effect of Other Parameters

The double chevron appearance on individual actin filaments is governed predominantly by the axial and azimuthal range of the cross-bridges, as long as the actin pitch or myosin repeat are not changed by >20% or so.

(*a*) Actin helix pitch. Rotating the actin filament or moving it axially causes axial movement of the positions at which the double chevrons appear but otherwise the visual appearance and the Fourier transform are essentially the same. Changing the pitch of the actin helix slightly changes the axial repeat between the chevrons, and thus changes the position of the peaks in the Fourier transform.

(*b*) Actin subunit repeat. This can be varied independently of the actin helix pitch. The calculations show that the fine details of the chevron pattern and long 115.9-nm repeat are affected slightly by choice of subunit repeat but good double chevrons and the transform were insensitive to this parameter.

(*c*) Varying the myosin subunit repeat has no effect on the axial spacings between the double chevrons and very little effect on their appearance. However, in the Fourier transform of the actin-bound chevrons, the spacing of the peak near 14.5 nm always corresponded exactly with the myosin repeat being modeled. Four other reflections (Table II, column c) also have positions that depend upon the myosin repeat.

(*d*) Weighting of the cross-bridge attachment. Changing the weighting of the choice for the cross-bridge does not move actin monomers in or out of range of a particular cross-bridge but only determines which actin monomer will be labeled if more than one is within range. The weighting thus does not affect the selection of which cross-bridges do attach to the surrounding actin filaments. The weighting seems also not to have any effect on the presence of the double chevrons, but the Fourier transforms do indicate that there is a change in structure. In the example shown in Fig. 7, changing the weighting from the center of the available range of the cross-bridge to one end changes the relative intensity of the 14.5- to 12.8-nm reflections.

(*e*) Two heads per cross-bridge. Reedy's original suggestion that the two heads from one myosin molecule might attach independently to two different filaments has recently been further investigated by Offer and Elliott (1978). We performed some modeling calculations with the types of weighting described above and found not unexpectedly that with our models the two heads tend to label adjacent actin monomers on the same filament. But with suitable choice of parameters we were able to simulate the binding of one two-headed cross-bridge to two actin filaments. We are still pursuing this class of model but we are concerned that with 6 myosin molecules per crown and only 4 of the 12 heads visible in each flared *X*, then at each level 8 of the 12 heads do not attach. We have not yet been able to invent reasonable rules which permit two myosin molecules to attach to two actin filaments each, while the other four molecules do nothing

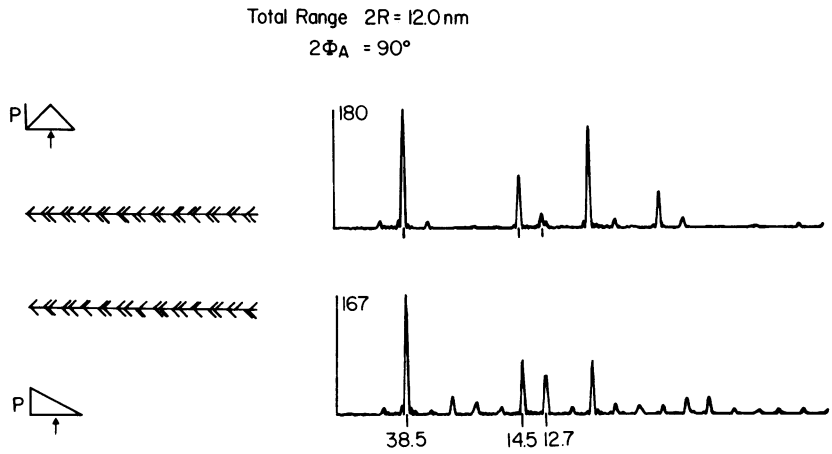


FIGURE 7 Diagram showing the effect of changing the weighting of which actin monomer will be labeled if more than one is within range. (*a*) The cross-bridge prefers to attach close to the center of the range. The probability of attachment is $P = \frac{1}{2} - \frac{1}{4}(|r|/R + |\phi|/\Phi_A)$. (*b*) The cross-bridge prefers to attach at one end of the range $P = \frac{1}{2} - \frac{1}{6}(|r - R|/R + |\phi|/\Phi_A)$. The weighting difference makes very little change in the double chevron appearance but makes a significant change in the intensity of the 12.8-nm reflection and transform.

at all. The problem could be simplified if we reverted to a two-stranded filament as described by Reedy (1968), but the available biochemical data (see Squire, 1977) make this an unlikely model.

DISCUSSION

By using a highly simplified model in which cross-bridges with one head directly face each actin filament at every cross-bridge level, our computer calculations have produced some initial models that simulate very pleasingly the double chevron structure, the flared *X*, and the long repeat seen by Reedy. The double chevron appearance on an individual actin filament turns out to depend on the range permitted to the myosin cross-bridges to reach along and around the actin filaments, and is independent of the exact axial or rotational orientation of the actin filaments. We find that the cross-bridge must be free to move 10–14 nm axially and be able to move about 90° around the actin filament.

It is interesting to note how similar our results are to a qualitative model published by Squire (1972). His Fig. 5 *b* showing where the cross-bridges attach to actin in rigor is very similar to our Fig. 6 (as indeed they should be as both figures are simulating the same set of data!). Squire constructed a model in which the myosin head could attach to actin only if the attachment did not require the head to distort >90°; he made no specific restrictions on the axial movement of the cross-bridges but he felt they were not likely to move more than 5 nm from their point of origin. Thus with our terminology he was using a model with $2R = 10$ nm and $2\Phi_A = 90°$, in excellent agreement with the values we feel give good models. The agreement between Squire's model and

ours is pleasing because the two experiments had different aims: on one hand, Squire was trying to show that Reedy's flared X could be produced by a six-stranded myosin filament (as opposed to the two-stranded model), and he included the myosin helix parameters in his model. On the other hand, we accepted that the myosin filament was six-stranded but we concentrated on finding out how free the cross-bridges are to move. The agreement of Squire's model with ours encourages us to think that we were justified at this stage in ignoring the helical parameters describing the myosin filament. Indeed, Squire (1972) has already pointed out that the exact form of the helix is not critical for the flared X pattern.

The values for the ranges R and Φ_A that give good double chevrons as assessed by eye (Fig. 3, Table I) confirm that the cross-bridges must be able to move to attach to actin in rigor. We cannot yet say whether the movement has occurred after the cross-bridges have attached to an actin passing very close to the ideal attachment position of the cross-bridge, or whether the cross-bridge can move this far before attaching. In view of the fact that the cross-bridges are probably attaching and detaching from actin even in rigor conditions (Marston and Weber, 1975), the latter possibility is more likely, but it is also possible that once a cross-bridge is attached, it may be pulled out of position by filament sliding and then become less able to detach. However, the value of 10–14 nm we find for the axial range of the cross-bridge in rigor is similar to the value of 8–12 nm for the active stroke in contracting muscle (Barden and Mason, 1978; Huxley and Simmons, 1973). The angular parameter Φ_A tells us that the attachment sites on the actin filament lie within an azimuth of about 90°. Since successive actin monomers along one of the two strands differ in azimuth by about 26°, the azimuthal restriction suggests that at each turn of the actin helix there are about four actin monomers per strand in each of these "target areas" (Reedy, 1968; Hill, 1975; Barrington Leigh et al., 1977). This may indicate that the cross-bridges are not flexible enough to reach completely around to the sides of the actin filament, or it could arise because the actin monomers not available to the cross-bridges are obscured by troponin molecules. Certainly if the troponin-tropomyosin repeat in insect is the same as in vertebrate (38.5 nm; Rome et al., 1973), then it exactly matches the insect actin helix pitch of 38.5 nm, so all the troponin molecules, sitting in opposed pairs, would always block sites at the same azimuth on the actin filament. With the actin filament situated at dyad positions in the filament lattice, the troponin molecules all sit at equivalent positions with respect to the myosin filaments, so that either all are in the way or none are in the way; if none, then no troponin molecule need every obstruct an actin monomer azimuthally accessible for force generation by a cross-bridge.

Although the axial and azimuthal range of the cross-bridges determines how many of the cross-bridges can attach to actin, these parameters do not alone control the complete distribution of links between the filaments. This distribution, and the appearance of the flared X, are determined also by the relative orientation of the actin filaments around the myosin, as had been inferred from electron micrograph appearances.

The Fourier transform of labeled actin filaments indicates that a period of 14.5 nm is expressed in the attachment of cross-bridges along any one actin filament. The exact

spacing of the reflection is always exactly the value given to the axial spacing of cross-bridge crowns on the myosin filament, so we cannot attribute this reflection to a spurious beat of the actin periods alone. We must conclude that the cross-bridges attach on average every 14.5 nm along the actin filament, although the actin filament structure only allows for attachments separated by whole multiples of 2.75 nm (e.g., 13.75 or 16.5 nm). It is intriguing that in our model (and possibly in practice, too) we see a myosin reflection from the transform of the actin filament! Thus in our models, and we imagine also in rigor muscle, the cross-bridges prefer to attach with as little distortion as possible as near as possible to their original positions on the myosin filaments, an average of 14.5 nm apart.

Further work is still to be done on the way the Fourier transform is affected by the parameters controlling the cross-bridge attachment, but the experimentally observed changes in the X-ray and optical diffraction patterns when ATP analogues are bound to rigor muscles (Marston et al., 1976; Barrington Leigh et al., 1973) make this an important area to study. The effects we have already noted of changing the pattern just by changing the weighting of attachment sites could well be related to some of the already reported changes induced by the analogues. Recently, a theory has been developed by Barrington Leigh et al. (1977) that explains rigorously the different reflections in the diffraction patterns from insect flight muscles. We feel that our modeling studies in real space complement their analysis of the diffraction patterns in reciprocal space and demonstrate some of their conclusions. At present there are two potentially useful conclusions to be drawn. (*a*) The cross-bridges attached to the actin filament can (and probably do) give rise to reflections normally attributed to the myosin filament. (*b*) The intensity of the 12.8-nm reflection (and probably other ones too) is influenced by the way in which the binding constant of myosin to actin is determined by the conformation of the cross-bridge. The binding of nucleotides to the cross-bridge could well affect the probability of attachment at different positions (see Fig. 7, for instance) as indeed suggested also by Barrington Leigh et al. (1977).

We plan in future papers to discuss the effects of arranging the cross-bridges helically on the myosin filament, of tilting the cross-bridges, and of computing two-dimensional transforms. We plan to approach the cross-bridge lattice in rigor vertebrate muscle in the same way.

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