BRIEF COMMUNICATION

MYOSIN SUBFRAGMENT-1 ATTACHMENT TO ACTIN

EXPECTED EFFECT ON EQUATORIAL REFLECTIONS

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ABSTRACT The characteristic equatorial X-ray pattern from a relaxed vertebrate skeletal muscle changes when the muscle is activated. In particular, there is a simultaneous decrease in the intensity of the first reflection (I_{10}) and increase in the intensity of the second (I_{11}). This observed change is almost reciprocal. When compared with the predictions of computer modeling, it produces a strong argument that the intensity change is due to a redistribution of myosin heads (myosin subfragment-1 or S-1), which results from the formation and configuration changes of actin-myosin links. Computer modeling shows that different actin-S-1 configurations will give different numerical values for I_{10} and I_{11}, assuming the same number of attachments. For a given configuration, the intensity changes are a nonlinear function of attachment number, so that direct scaling of force to reflection intensity may be difficult. Data from active muscle are consistent with the notion that in different states of active muscle, i.e. shortening or isometric, there are different average configurations of actin-myosin attachment and different numbers of actin-myosin links.

The equatorial X-ray reflections from striated muscle provide a direct way of observing gross molecular rearrangement in activated muscle. For vertebrate skeletal muscle, the intensities of the first two reflections, the 10 and 11, vary in a characteristic way when the muscle changes state from rest to activated to rigor (1, 2). The intensity change is thought to be caused by movement of myosin subfragment-1 (S-1) moieties away from a relaxed arrangement around thick myosin filaments towards thin, actin-containing filaments. I wish to characterize this movement to describe the molecular changes that actually generate muscle tension.

The nature of the movement by some of the S-1's has been variously described as radial or azimuthal (3, 4). Neither of these descriptions by itself seems to explain adequately recent experimental data, in which the intensity of the 10 (I_{10}) decreases while the intensity of the 11 (I_{11}) increases when muscle is fully activated (5, 6). The difficulty in explanation arises because the X-rays are looking not at the vectors individual S-1's follow but rather at the distribution of all the S-1's.

The calculations presented in this report show that if some of the S-1's become dis-

TABLE I

EFFECT OF ACTIN-MYOSIN LINKS ON I_{10} AND I_{11}

Total S-1 attached to actin	I_{10}*	I_{11}
%		
Configuration A‡		
0	26	15
10	21	22
20	17	31
30	13	41
40	9.3	53
50	6.5	67
Configuration B‡		
0	26	15
10	23	18
20	20	21
30	17	25
40	15	29
50	12	33

*Intensities are scaled to $|F_{00}|^2 \times 10^{-3}$. The form factor used for calculations is basically that reported earlier (9).

‡Configuration A is similar to the rigor reconstruction of Moore et al. (10). Configuration B is attached more perpendicularly to the actin filament, both axially and azimuthally.

For the highly unlikely case of 100% S-1 attachment, the calculated intensities are $I_{10} = 4 \times 10^{-4}$ and $I_{11} = 147$ for configuration A, and $I_{10} = 3$, $I_{11} = 57$ for configuration B. Since it is real, $I_{10} \geq 0$ in all cases. Thus a plot of I_{10} versus attachment number will almost always be markedly curved, while a plot of I_{11} versus number may closely approximate a straight line, provided the increase in intensity is relatively small.

tributed around the actin filaments, the intensity of the 11 reflection will increase and the intensity of the 10 reflection will decrease. The simplest explanation for such S-1 distribution around an actin filament is that some S-1's have become attached to actin. The calculations also show that the configuration of attachment will strongly influence the intensities of the reflections.

The exact vector a particular S-1 will follow to become attached will depend on how the myosin is packed into the thick filament, which is not yet known. But published calculations indicate that for three- or four-stranded thick filaments the surfaces of many of the myosin S-1's are within 20 Å of actin surfaces (7). Since this distance is of the same order as the indicated thermal movement by the S-1's,[1] there may be no need to postulate a change in the myosin thick filament that brings all the S-1's near actin.

In relaxed muscle, all the S-1's are presumed distributed about the myosin filament. When the muscle is activated, some S-1's become attached to actin, creating a second center of S-1 distribution. The S-1's attached reflect coherently with the actin, causing

[1]One can obtain an estimate of the temperature effect by comparing the experimentally observed decrease in intensity with the decrease calculated for different models as one moves from the origin. For example, in Fig. 3 of Huxley and Brown (8), comparison of the observed distribution and predicted distribution for model *b* yields a temperature factor of 3.9×10^4 (Å^2), corresponding to a mean square amplitude of vibration of 22 Å in the equatorial plane. This estimate of vibration by the myosin heads in the radial and azimuthal directions should be treated with caution.

an increase in I_{11} and decrease in I_{10}. The exact quantitative effect will depend on the fraction of total S-1 that binds to actin and the configurations of attachment. The form factor for the calculations is basically that reported earlier (9).

This dual effect is illustrated by the results given in Table I, for two configurations of actin–S-1 complex and various proportions of attachment. Configuration A represents a rigor complex similar to reconstructions from electron microscopy and previous modeling (10, 11). In configuration B, the S-1 is almost perpendicular to the actin helix, axially and azimuthally. For both structures, I_{10} decreases and I_{11} increases monotonically with increasing attachment number. The data are plotted in Fig. 1 as the ratio I_{11}/I_{10}. It should be noted that for both configurations the plot is curved, which may make direct scaling of force and intensity ratio difficult. This is a general

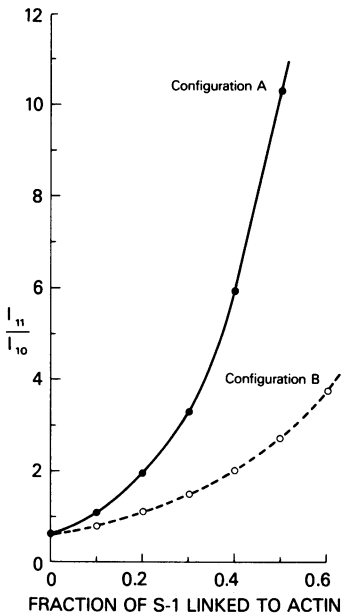

FIGURE 1

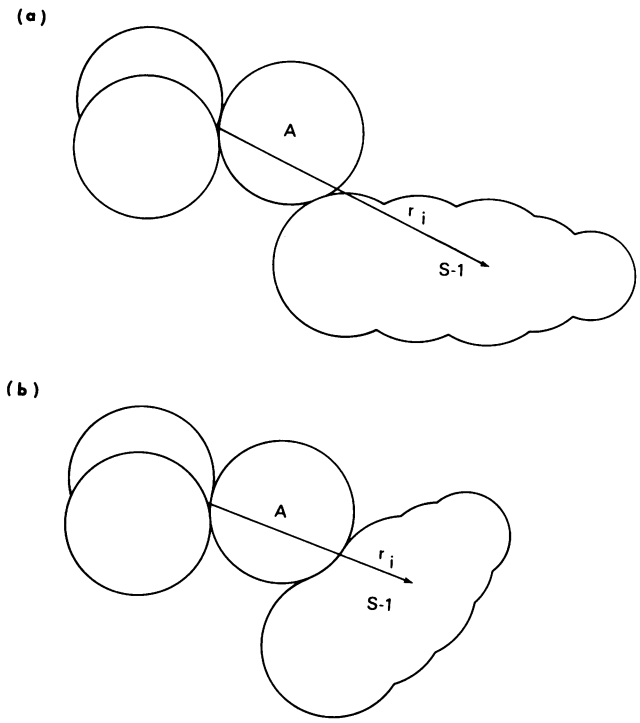

FIGURE 2

FIGURE 1 A plot of the intensity ratio I_{11}/I_{10} against the proportion of myosin subfragment-1's attached to actin. Configuration A is similar to a rigor complex, while configuration B is more perpendicular to the actin helix.

FIGURE 2 Equatorial projections of two possible actin-S-1 structures. Using cylindrical coordinates, r_i is the distance from the origin of the actin helix to a specific region of the S-1 molecule. This view is equivalent to looking down the actin helix. (*a*) A perpendicular structure, similar to configuration B. (*b*) A possible rigor structure, different from that in *a*, in that it has slued around the actin helix both axially and azimuthally. Both these movements bring the S-1 mass closer to actin, reducing the value of r_i as compared with *a*.

point, though the degree of nonlinearity in an I_{11}/I_{10}-number plot depends on configuration. Further, in the region of low attachment number (0–30%), the plots approximate straight lines. The important point is that the two curves are different, showing that there is no direct relation between intensity ratio and attachment number. Indeed, changes in configuration of attachment can cause changes in I_{11}/I_{10} that overshadow the effect of attachment number.

The actin contribution to I_{10} and I_{11} is primarily described by zero-order Bessel coefficients, $J_0(2\pi r_i R)$ (9). Because of this, the azimuthal and axial coordinates of attachment configuration are important only insofar as they bring the mass of the S-1 closer to the actin helix or further away. Thus a tilting mechanism after attachment, as has been postulated for active muscle (12–14), could cause a further increase in I_{11}/I_{10} without additional attachments being made.[2]

Fig. 2 illustrates the equatorial view of a possible change in configuration subsequent to actin-myosin attachment. Fig. 2 *a* is a view down the actin helix of three actin monomers, one monomer being decorated with an S-1 in a configuration with no axial tilt. The S-1 can now be tilted azimuthally, within the plane, and axially, out of the plane. Such a change could produce the projection seen in Fig. 2 *b*. The S-1 mass has moved closer to the actin helix, as indicated by the arrows r_i. Such a change will cause an increase in I_{11} and decrease in I_{10}.

It is also clear from Fig. 2 that a configuration change that does not alter the equatorial projection will not be seen by the equatorial reflections. That is, if an S-1 is tilted axially by, say 45° with respect to the perpendicular, a shift to −45° will produce the same equatorial pattern.

The variation of intensities with attachment number and configuration can be applied to results reported for active muscle (5). When resting muscles were activated at constant length (isometric), I_{10} went down relative to rest, while I_{11} increased. When the muscles were then allowed to shorten against constant load (isotonic, about 0.14–0.4 maximal force), the intensity of both reflections increased slightly.

The data can be explained by saying that in isometric tetanus, 40% of the S-1's are attached, all perpendicular to the thin filament (configuration B) (I_{10} = 15, I_{11} = 29, cf. Table I). If against half-load, 20% of the S-1's are attached, all in rigor complex A, then I_{10} = 17, I_{11} = 31, and both intensities would have increased. This is consistent with theories of muscle contraction in which there are fewer actin-myosin links in an actively shortening muscle than an isometric tetanus (15).

Alternatively, one could have the cross-bridges in a very angled configuration in iso-

[2]An interesting corollary of this is that any change which brings mass closer to the actin will cause an increase in I_{11} and decrease in I_{10}. In particular, a rolling of tropomyosin in the actin groove may cause a slight (10–20%) increase in I_{11}/I_{10}, which may have a different time-course than S-1 attachment. There is some experimental data from glycerinated rabbit psoas muscle (17) which suggests a change in attachment configuration without (much of a) change in attachment number. Those equatorial data, in the presence of adenylylimido diphosphate, could be explained in terms of Fig. 1 as a vertical arrow from configuration A to configuration B. This would cause a decrease in I_{11}/I_{10} without altering attachment number.

metric tetanus, but perpendicular in shortening muscle, thereby requiring an increase in attachment number to explain the data (16).

Thus the modeling shows that a simultaneous change in the number of attachments and the average configuration of attachment is consistent with the experimental data comparing actively shortening muscle with isometric tetanus. The original paper (5) suggested that the number of attachments remained essentially constant, while the average configuration changed. This could be true for the case mentioned in the discussion of Fig. 2, where a second configuration is a mirror image of the first and has the same equatorial projection.

In summary, the reciprocal decrease in I_{10} and increase in I_{11} seen when skeletal muscle is activated is a very strong argument that the observed change is due to a redistribution of S-1's resulting from formation and configuration changes of actin-myosin links. This argument is valid for different symmetries of the myosin filament (e.g. two-, three-, or four-stranded packing) though the details vary. Since there is considerable thermal movement by the S-1's in a relaxed muscle, an active movement by S-1's before attachment to actin is not needed, though it may exist. A different way of stating this is that in a relaxed muscle, the S-1's form a diffuse equatorial halo around the thick filament. When the muscle is activated or goes into rigor, some of the mass from this diffuse halo congeals at the actin filaments. The obvious biochemical cause for this coalescence is the formation of actin–S-1 links. Different actin–S-1 configurations give different numerical values for I_{10} and I_{11}. For a given configuration, the intensity changes are probably a nonlinear function of attachment number. A plot of I_{11}/I_{10} versus attachment number may nonetheless approximate a straight line when the fraction of attachments is in the range 0–30%. Data from active muscle are consistent with the notion that in different states of active muscle, there are different average configurations of actin–S-1 attachment.

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