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Recent X-ray diffraction studies of muscle contraction and their implications

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Recent studies on the interference fringes in the myosin meridional reflections provide a new source of structural information on cross-bridge movement during mechanical transients and steady shortening. Many observations can be interpreted satisfactorily by the tilting lever-arm model, with some assumptions, including the presence of fixed repeating structures contributing to the M3 and higher-order meridional reflections. In isometric contraction, the lever arms are oriented near the start of the working stroke, with a dispersion of *ca* ± 20–25°. Upon a rapid release of 10–12 nm, they move to the end of the stroke, with a well-known T2 delay of 1–2 ms. This delay must represent additional processes, which have to occur even in tension-generating heads, or activation of attached heads, which initially do not develop force. Surprisingly, in muscles shortening at moderate loads (0.5–0.6 P_0), the mean position of the heads moves only 2–3 nm closer to the M-line than in the isometric case, reminiscent of the Piazzesi–Lombardi model.

Keywords: muscle X-ray diffraction; interference; cross-bridge

1. INTRODUCTION

The current generation of electron–positron storage rings provides X-ray beams whose characteristics are particularly well suited to the detailed study of transient events in muscle contraction. Owing to the very small source size (*ca* 200 μm), the focused X-ray beam, at the detector of a 6 m long camera, can be less than 100 μm in height. This gives extremely high order-to-order resolution in the X-ray patterns (*ca* 60 000 Å), and makes it possible to record very informative fine structure in the meridional reflections from contracting muscle. Moreover, the very high X-ray flux (10^{13}–10^{14} photons per second) make it possible to record such patterns with millisecond time resolution or better.

Recent studies of interference effects on the meridional reflections from the myosin filaments have revealed important new details about the configuration of the myosin heads and their changes during rapid mechanical transients (Linari *et al.* 2000; Lombardi *et al.* 2000; Huxley *et al.* 2000–2004; Piazzesi *et al.* 2001, 2002, 2004; Irving *et al.* 2002; Reconditi *et al.* 2003). These effects arise because of interference between the diffraction from the two halves of each thick filament. All the thick filaments have exactly the same construction, so that the centres of scattering mass of the axial arrays of myosin heads, in the two halves of each filament, have exactly the same axial separation in each filament. Therefore, each one gives the same interference fringes on the reflections that arise from the underlying axial repeat of the cross-bridges in each half filament. The position of the interference fringes on the first myosin meridional reflection (M3) at 14.56 nm in contracting muscle provides an extremely sensitive measure of the changes in the axial position of the centre of scattering mass of actin-attached myosin heads. It is capable of detecting movements of 1–2 Å, as first recognized by Linari *et al.* (2000). Measurement of the ratio of the intensities of the two major peaks, produced by the sampling of the M3 reflection, is a convenient way of measuring the relative movement of the sampling fringes and, hence, of the myosin heads. In practice, we analysed the synchronized changes, during very small, rapidly applied decreases in muscle length (Huxley & Simmons 1971), in terms of the sliding-filament, tilting lever-arm model, with the catalytic subunit of myosin S1 attached to the actin filament, whose axial sliding, relative to the myosin filament backbone, is produced by a change in angle of the 'lever arm', i.e. the elongated domain of myosin S1 connecting the catalytic subunit to the S1–S2 junction.

This type of model gives a good qualitative account of the changes actually seen, but certain important assumptions have to be made for the agreement to be reasonably quantitative (Huxley *et al.* 2001; Piazzesi *et al.* 2002).

2. ASPECTS OF THE MODELLING

(a) When the predictions of this model were compared with the ratio changes actually observed, it was found that the ratio changed only by about half the expected amount during small releases (1 or 2 nm per half-sarcomere). Also, instead of the ratio progressively decreasing to almost zero with larger releases of 6–8 nm (as would be expected if the outer fringe reached the first zero of the envelope of the diffraction peak), the ratio saturated at a value of about 0.25–0.30. This is readily explained by the presence of some fixed component in the isometric muscle, perhaps unattached partners of the attached heads, with approximately the same scattering contribution as the attached

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heads. Initially, the total centre of mass movement is reduced by a factor of two. Then, as the lever arm tilts further, the axial profiles of the catalytic subunit, and of the lever arm, become increasingly misaligned. Consequently, the scattering contribution of the moving head is progressively reduced, while that of the fixed component remains constant. Therefore, the movement of the centre of effective scattering mass of the head and the fixed component combined slows down and eventually stops, and then reverses, as the lever-arm angle continues to tilt. These effects can be computed, and conform well to experimental observations (Huxley *et al.* 2001; Piazzesi *et al.* 2002).

The identity of the additional fixed, diffracting component has not been firmly established. One possibility is that the 'second' unattached head of these myosin molecules, whose 'first' head is attached to actin, is stabilized in some way by its partner, with its lever arm oriented near to the position where catalytic subunit and lever arm are maximally aligned in projection onto the filament axis. In isometric muscle, the attached head has its lever arm tilted 5–10°, or more, beyond this position, i.e. further away from the rigor position (assumed to correspond to the end of the working stroke). This arrangement provides good modelling of the initial increase in intensity with smaller releases (as the two heads become aligned), and gives a competent match to experimental observations, both of ratio and total intensity variation, with the extent of release.

Although the fixed component could, in theory, be the 'second heads', there is no over-riding reason why this should be the case, and there are several reasons why the situation may be a lot more complicated.

(i) If the fixed component of the M3 reflection is produced only by the 'second heads', then, for their contribution to be approximately equal to that of the attached heads in isometric contraction, the dispersion of their lever arms *about the S1–S2 junction* must be exactly the same as the dispersion of the lever arms of the attached heads. This seems an unlikely coincidence.

(ii) The position of the interference fringes on the M6 reflection (at 7.28 nm) in isometric contraction is entirely different from what would be the case if this reflection was largely the second-order diffraction from the myosin heads giving the M3 reflection. Instead, the reflection must come mainly from some other structure, very possibly in the thick filament backbone. The same is true of the M9 and M15 reflections. If the backbone gives reflections at these submultiples of the 14.56 nm spacing, it is extremely probable that it would also give a contribution at the 14.56 nm fundamental spacing, and contribute to the fixed component there.

(iii) Polarized fluorescence studies of lever-arm orientations in contracting single fibres indicate that, at any one time, only a small fraction (10–20%) of all the myosin heads is involved in tension development (Hopkins *et al.* 1998, 2002). There is, therefore, a very large population (80–90%) of unattached heads. These are liable to make a significant contribution to the diffraction pattern, even if they are maximally disordered by lever-arm rotation about the S1–S2 junction.

Nevertheless, provided the sum of these various possible factors supplies a fixed contribution to the M3 periodicity with the appropriate amplitude and phase, then, the behaviour of the M3 reflection is accurately explained by the model.

(b) While rationalizing the behaviour of the M3 reflection is a necessary condition for the plausibility of a model, it is not a sufficient one. Because the whole X-ray diffraction pattern can be computed from the known high-resolution three-dimensional structure of the myosin head, it is also incumbent on a model to account for the characteristics of the higher-order myosin meridional reflections.

3. HIGHER-ORDER REFLECTIONS

The most prominent is the M6 reflection. Thus, it is immediately obvious that other components must contribute to this, because, during isometric contractions, the relative intensities of the two interference peaks on this reflection are the reverse of what would be predicted by the original model, with or without the second component. That is, the predicted phase is wrong. The incorporation of an additional 7.25 nm component alone, to correct this, is insufficient to explain the behaviour of the M6 reflection during the quick releases (whose effect on M3 has been correctly predicted by the two-headed model). The M6 reflection increases in intensity by only 40–60% during such releases (the heads alone would give an increase three or four times as great), but, if a sufficiently large fixed component is incorporated to reduce the predicted percentage increase to the observed values, the predicted isometric intensity is six or seven times too large. Basically, this is because the predicted contribution of the simple two-headed model to the M6 reflection is much too large relative to the intensity of the M3 reflection.

Fortunately for the modelling, this discrepancy can be corrected in a very simple way (Huxley *et al.* 2002, 2003), by allowing considerable dispersion of lever-arm angles to be present. It has been apparent, for some time, that the lever arms of all the attached heads are not oriented at exactly the same angle, owing to the differing axial and helical repeats in the actin and myosin filaments. Indeed, asynchrony of cross-bridge action is a very desirable feature of the basic sliding-filament model, because it ensured that tension would develop smoothly during shortening. Irving *et al.* (2002) have pointed out that a dispersion of ±2 nm in the axial position of the catalytic domains would allow axial alignment of a high proportion of them with actin monomers, which can always be found within an axial distance of 2.75 nm, because their axial repeat is 5.5 nm. This argument does not consider the helical structure of the filaments. Nevertheless, given that the S2 connection to the myosin filament backbone could provide radial and azimuthal flexibility, and the likelihood that only a small proportion of heads are involved in force production at any given moment (Hopkins *et al.* 1998), such a dispersion would probably be sufficient to allow attachment. Such an axial dispersion corresponds to a dispersion of about ±12° in lever-arm angles. Irving *et al.* (2002) show that this would have little effect on the predicted variation of M3 intensity (relative to the isometric value) with average lever-arm angle, when modelled as a Gaussian dispersion with this characteristic width. We have found that a uniform dispersion of at least ±20° is needed to reduce the total isometric intensity of the M6, predicted by the model, to the observed value, if a sufficiently large fixed component is present to reduce the maximum percentage intensity increase, during quick releases, to its observed range. If the dispersion is larger than ± 25°, then the predicted M6 intensity increase, during quick releases, takes place far too slowly, even though the same maximum is reached. Uniform dispersions of ±20° and ±25° give a variation of intensity increase, with extent of release, which approximates the observed behaviour. A value of ±23° gives the best agreement (Huxley *et al.* 2003). Gaussian dispersions, with *ca* ±19° standard deviation, give predictions that approximate the observed intensity variation with average lever-arm angle (i.e. with extent of release), but less satisfactorily than the uniform dispersions.

Even such large dispersions still give a satisfactory account, not only of the variation of M3 intensity with extent of release, but also of the change in the relative intensities of the two interference peaks in that reflection. Indeed, the agreement is also somewhat better than that given by the models without dispersion.

4. IMPLICATIONS OF THE MODELLING

We define the lever-arm axis as the line joining residue 707 (pivot point) to residue 843 (tip of lever arm). To explain the observed small increase in total M3 intensity for small releases, it is necessary to assume that the isometric lever-arm angle lies slightly beyond that at which maximum alignment of lever arm and catalytic subunit occurs, and gives maximum intensity (Irving *et al.* 1995). The latter occurs at an angle of 48° away from the rigor position we have used, and *ca* 46° away from a recently determined rigor position (Holmes *et al.* 2003). This is equivalent to 34° from the Rayment *et al.* (1993*b*) position. In the 23° dispersion model (both heads dispersed), we could account for the observed intensity increase of 10–15% with the unattached 'heads' at 48° average lever-arm angle, and the attached heads at an average angle of 63° away from our rigor position. Other models can, probably, be devised with slightly different parameters, but they must all share the same basic feature, i.e. that the attached heads be concentrated in positions near to the beginning of the working stroke, and, on average, *ca* 10 nm away from the rigor position. The rigor position is usually assumed to correspond to the end of the working stroke, and, indeed, the total intensity of the M3 reflection, and the relative intensities of the interference peaks do continue to change, in the predicted manner, for releases corresponding to *ca* 10 nm of relative filament sliding.

This concentration is an interesting result, because, in the simplest model, it might have been expected that an isometric contraction would include cross-bridges at all stages of the working stroke, up to the point at which they detached. It is possible, however, that the probability of detachment is significant throughout the stroke, though smallest at the beginning. It is also possible that most heads, in the later stages of the working stroke, detach as the isometric state becomes established, while others attach and remain near the beginning. We observed some time ago (Huxley *et al.* 1983) that the M3 reflection changed very little in intensity during slow speed shortening (at 0.96 P_0), so, presumably, the later stages of the working stroke were also unoccupied at that time. In later experiments (unpublished), we recorded the characteristics of the interference fringes on the M3 reflection during moderate speed shortening (0.57±0.02 P_0), and observed that the change in intensity ratio from isometric is relatively small (0.27±0.07), corresponding to an axial shift of average catalytic subunit position of the attached heads by only *ca* 2–3 nm. Reconditi *et al.* (2004) also observed that, during isotonic release from isometric contraction, the behaviour of the interference peak ratio indicated detachment after a smaller sliding distance at high load than at low loads.

Reconditi *et al.* (2004) calculated that the compliance of the myosin head itself is only 1.4 nm T_0^{-1}. Thus, it cannot be assumed that the basic internal mechanism within the myosin head is going through the same motions as it does at very low load (when up to 10 nm of axial movement of the end of the lever arm takes place, relative to the catalytic subunit), but that the motion is being absorbed by a large elastic element.

Another possibility is that most of the heads, contributing to and dominating the X-ray pattern, and moving during a quick release, are not yet developing tension, and detach quickly during slower shortening, and quickly re-attach again near their starting positions. The polarized fluorescence measurements, however, show that only a small proportion of all the heads (*ca* 10%) are attached and move during such quick releases, so that, if most of them were inactive (say 80%), then the force generated by each of the remaining active heads would need to be implausibly high, about 50 pN.

Several difficulties arise from these observations. If the head detached from actin halfway through its maximum stroke, it is difficult to see how the system can avoid wasting a substantial amount of the available energy. From the quick release experiments, it is apparent that the heads can develop force all the way to the end of their stroke. Therefore, it is difficult to think of an actual mechanism that would channel this energy into useful work earlier in the stroke under a larger load, or would enable a head to repeat the early part of the stroke, and do more work. Nevertheless, it is interesting that the two-pathway model of Piazzesi & Lombardi (1995) leads to distributions not unlike those observed.

Also, the available information (Woledge *et al.* 1985; Huxley 2000) indicates that *ca* 11.5 *k*T of energy should be available from ATP to perform mechanical work (to account for the 60%, or more, of the total energy that can appear as work). This energy is equivalent to *ca* 45 pN nm^{-1} of work, or 9 nm of movement, against a peak force of 10 pN, if the force decreases linearly with distance moved. In the isometric distributions, the average distance to the rigor position that we see is *ca* 10 nm. So, unless current estimates of peak cross-bridge force are much too small, then most of the stroke is needed to account for the work output.

It will be interesting to see how this problem is resolved.

5. IMPLICATIONS OF THE T2 TIME CONSTANT

Huxley & Simmons (1971) showed that, after a small, rapid release, tension redeveloped with a time constant of 1–2 ms. Because there is insufficient time for a significant number of myosin heads to attach or detach, it was concluded that this time constant represents the rate at which some process occurs within already-attached heads. In skeletal muscle, there is no evidence, yet, that the cross-bridge stroke occurs in more than one step. Consequently, if a myosin head is already developing force, it should be essentially able to go to the end of its stroke instantaneously, and function as a pure elastic element. Nevertheless, to explain the T2 delay, we need a relatively slow-rate process. Either there is, after all, an initial step, where force is developed before the second step of lever-arm movement, and that second step represents the observed rate constant (not unlike the original Huxley–Simmons model), or, alternatively, there is a population of attached heads, which are not yet developing force, and it is the activation of those heads that provides the rate constant. This activation could depend on lever-arm angle, and could be accelerated by tilting inwards, from the angle of initial attachment, during a quick release. The total movement of an attached cross-bridge could, then, be considerably greater than that over which it develops force. Additional factors are the forces, positive and negative, developed by attached pre-stroke heads, and their behaviour when the equilibrium is disturbed by a sudden length change; similar considerations apply to the overall distribution of post-stroke heads (Chen & Brenner 1993).

So there are still some interesting mysteries to be solved in the cross-bridge mechanism, even at a relatively simple level.

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