Journal of Muscle Research and Cell Motility **6**, 153–161 (1985)

Crossbridge behaviour during muscle contraction

H. E. HUXLEY and M. KRESS

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

Received 20 December 1984 and in revised form 29 January 1985

Summary

A number of recent observations by probe and X-ray methods on the behaviour of crossbridges during contraction is considered in relation to the energetics of the process. It is shown that a self-consistent picture of the crossbridge cycle, compatible with these observations and involving strongly and weakly attached crossbridges, can be obtained providing that the tension-generating part of the crossbridge stroke is only about 40 Å i.e. about one-third of the usually accepted value. The myosin head subunits in the tension-generating bridges could have a configuration close to that of rigor. A mechanism is suggested whereby rapid tension recovery after quick releases up to 120 Å could still be produced by such a system.

Introduction

The purpose of this note is to draw attention to a relationship which may exist between a number of recent observations concerning the behaviour of crossbridges in vertebrate striated muscle during the tension-generating process. The observations are interpreted in terms of a moving crossbridge model with a tension-generating stroke much shorter than has usually been supposed, thereby allowing the tension-bearing myosin heads to adopt a configuration close to the rigor one by extension of elastic elements; other myosin heads are attached to actin in mobile and more weakly bound states.

Observations

(1) According to electron paramagnetic resonance (EPR) measurements, (Cooke *et al.*, 1984) about 20% of myosin heads in an isometrically contracting muscle are immobilized on a submillisecond time scale.

0142–4319/85 $03.00 + .12

(2) Other EPR measurements (Cooke *et al.*, 1982) indicate that the spin labels on the immobilized myosin heads have an orientation within $\pm 10^\circ$ of that seen in rigor.

(3) Polarized fluorescence observations using εATP (Yanagida, 1981) show that in an isometrically contracting muscle fibre, in the presence of up to 0.1 mMεATP, all the fluorescent labels have an orientation within 2° of that seen in rigor muscle with εADP.

(4) Dichroic measurements using a different label, attached to SH_1 (Burghardt *et al.*, 1983), also indicate that there is a fixed orientation of the labels during isometric contraction, but in this case the orientation is different to that observed in rigor.

(5) X-ray diffraction observations on the equatorial reflections from isometrically contracting muscles indicate that up to 90% of the myosin heads are in the close vicinity of the actin filaments (Haselgrove & Huxley, 1973; Yagi *et al.*, 1977; Huxley, 1980). The equatorial intensities have changed most of the way from those characteristic of the relaxed state towards those found in rigor, and other evidence from three different types of experiment (Cooke & Franks, 1980; Thomas & Cooke, 1980; Lowell & Harrington, 1981) all indicates that in vertebrate striated muscle in the rigor state, virtually all the myosin heads are attached to actin. Since the changes in the equatorial pattern in an activated muscle only take place when there is overlap between the actin and myosin filaments, it seems very likely that these changes are brought about by attachment of myosin heads to actin. It is possible, however, that attachment of one S1 head of a myosin molecule could keep both heads close to the actin filaments. If this took place on a random basis, then 60% attachment of individual myosin heads could keep 84% of myosin head pairs close to actin by either double or single attachments.

(6) The time course of the change in the equatorial X-ray pattern during contraction shows a substantial lead over the time course of tension development (Huxley, 1975). This indicates that at early times during contraction (and perhaps at later ones too) there are a substantial number of attached myosin heads which are not developing tension.

(7) During shortening of a muscle at moderate speed (for example, under conditions where the tension generated is half maximal) little change is seen in the equatorial X-ray pattern as compared to that observed during isometric contraction (Podolsky *et al.*, 1976; Huxley, 1979). Perhaps even more strikingly, when a quick release is applied to an isometrically contracting muscle, sufficient to reduce tension to a low value for 10 ms or more, no change in the equatorial pattern towards that characteristic of relaxed muscle is seen (Huxley *et al.*, 1983). These observations indicate that in an active muscle a high proportion of the crossbridges are always to be found in an attached state, even in circumstances where the number of tension-generating bridges is substantially reduced.

(8) X-ray diffraction observations on the layer line reflections from contracting frog muscles show only minimal signs of 'labelling' of the low order actin reflections by myosin heads (Huxley *et al.*, 1982). Any increase in intensity, for example on the first actin layer line, appears to be less than 5–10% of the value seen in rigor muscle. Thus the number of myosin heads attached to actin in a specific position and configuration is likely to be not more than 20–30% of those attached in rigor, because of the square law relationship between number of diffractors and intensity ($0.3^2 \fallingdotseq 0.1$). Observations of a

component of the intensity increase on the 59 Å layer line specifically associated with tension development does confirm that some helically ordered attachment takes place during contraction (Huxley *et al.*, 1985; see also Matsubara *et al.*, 1984).

(9) Quick release experiments on single muscle fibres (Huxley & Simmons, 1971; Ford *et al.*, 1977) indicate that crossbridges remain attached to actin over a total relative sliding distance between actin and myosin filaments of about 120 Å. However, the results do *not* require that a given crossbridge develops tension over the whole of this distance (see below).

(10) The quick release experiments also show that the crossbridges behave as if they contained an undamped elastic element in series with an element which developed tension and could shorten. Under maximal isometric tension, the elastic element would be stretched by 30–40 Å (Ford *et al.*, 1981).

(11) If it is assumed that (a) there is an efficiency of not more than 50% for the conversion of chemical energy into mechanical work in a muscle, (b) one ATP molecule is hydrolysed during one cycle of action of one myosin head and (c) the maximum free energy available is 50 kJ mol^{-1} (Kushmerick & Davies, 1969) then one can estimate the number of crossbridges which must go through one cycle when the muscle shortens a given distance against a given load. It can be calculated that during very slow shortening at almost maximum load, at least 53% of all the myosin heads must split one molecule of ATP for every 120 Å of relative sliding movement. Similar values can be derived from the known ΔH changes and heat production in muscle (Curtin, personal communication). These estimates are based on (i) a value of 3 kg cm^{-2} for the maximum isometric tension exerted by a single fibre from a frog muscle, with 15% of the cross-sectional area occupied by nonfibrillar structures, (ii) a side-spacing of 425 Å for the myofilament lattice, (iii) 49 repeats of the 143 Å axial periodicity of the myosin heads per half sarcomere, and (iv) three myosin molecules (six heads) for each 143 Å repeat.

(12) From the same calculation it follows that if a myosin head developed tension over its entire 120 Å attached 'stroke', then at any given time 53% or more of all the myosin heads would be developing tension. However, if a myosin head developed tension over a smaller part of its attached stroke, say 40 Å, then at any given time a correspondingly smaller proportion of heads would be developing tension; in this instance, about 18% of the total. However, the same *total* number of myosin heads (53%) would go through the cycle during 120 Å of filament sliding.

Interpretation

If we are to fit the observations summarized above into a satisfactory picture, we have to arrive at a self-consistent set of values for (a) the length of the *active* working stroke (defined as the distance over which an attached crossbridge develops tension), (b) the proportion of crossbridges attached and developing tension, and (c) the proportion of crossbridges attached to actin and not developing tension. That is, the values must be

reasonably compatible with all the different types of measurement mentioned above. It is conceivable that some of the measurements may be misleading; for example, in some of the probe studies, ATP-diffusion problems may have left a small rigor core in a few of the contracting muscle fibres, despite all the precautions taken to eliminate this possibility. However, for the purpose of the present discussion we will accept the published conclusions and see whether they will fit into a consistent picture.

If we accept the EPR value of 20% of myosin heads firmly attached to actin, and if we presume that all or most of those are developing tension, and that no other ones are, then we must limit the active working stroke to about 45 Å. This is because, with a short working stroke, the tension generated by an attached crossbridge is larger (for a given energy use during the stroke) than it would be for a long working stroke. So, for a given total tension, fewer crossbridges need to be pulling at any given instant. This would be consistent with the X-ray results if there were two classes of attached crossbridges: (i) firmly attached ones, bound to a specific site on actin, and having a limited range of defined configurations; and (ii) loosely attached ones, bound at a specific site but able to move fairly freely and rapidly over a considerable range of orientations. If these latter crossbridges could adopt a range of internal configurations they would appear mobile and disordered to the EPR measurements. The 20% firmly attached bridges, even if all identically oriented, might only contribute 4% of the intensity of rigor-type labelled actin first layer line reflection. [This is because the *amplitude* of their contribution would be 20% (0.2) of their contribution in rigor; if the contribution from actin itself was very small, the intensity ratio would be the square of this $(0.2 \times 0.2 = 0.04)$]. The remaining attached crossbridges would contribute little to the layer line pattern because of their disorder. However, all of the crossbridges which were attached to actin would contribute to the equatorial pattern, because they would still give rise to extra density near the actin filaments regardless of their axial and azimuthal disorder. Thus there could be a further 40% or more of weakly attached myosin heads, as evidenced by the equatorial reflections.

This concept of there being two classes of attached crossbridges contributing in different ways to the X-ray diffraction diagram would fit in well with points (6) and (7) above. During initial tension development, most crossbridges would first bind to actin in the weakly attached state, producing an equatorial change but no tension. When the muscle had become fully active, there would still be a large number of weakly attached bridges (if only a proportion of them could change to the strongly attached state at a given time) as well as others developing tension, and this would still be the case during moderate speed shortening if attachment to the weakly bound state was quite rapid. Indeed, this attachment rate might be very high in a fully active muscle if an attached head facilitated the reattachment of its partner. Thus the transition from isometric contraction to moderate speed shortening would not be accompanied by any substantial changes in the equatorial X-ray pattern. The number of strongly attached bridges (and hence the tension) would undergo a substantial decrease, but the total number attached could stay relatively constant.

The weakly attached crossbridges might have some similarities to the attachments seen at low ionic strength in relaxed muscle fibres (Brenner *et al.*, 1982), and in solution experiments (Chalovich & Eisenberg, 1982). The possible significance of the low ionic strength attachments in the crossbridge cycle has been discussed by Matsuda & Podolsky (1984). However, there are some significant differences in behaviour between such attachments and the weakly attached crossbridges of physiologically active muscle which may arise from biochemical and structural differences in the systems. First, the attachment in the weakly bound state which we envisage happening in intact muscle under normal physiological conditions is controlled by the activating mechanism and only takes place when the thin filaments have been switched on by the calcium–troponin–tropomyosin system (Huxley *et al.*, 1984). Second, even when the filaments are switched 'on', initial attachment follows with a significant time delay (about 10 ms), which would not be predicted by the very rapid equilibrium of the binding seen at low ionic strength. Third, both the timing and the extent of the decrease in the intensity of the myosin layer line pattern during contraction (Huxley *et al.*, 1982) indicates that the formation of weakly attached crossbridges under physiological conditions is not compatible with the preservation of a helically ordered myosin filament structure, in contrast to the situation in relaxed muscle at low ionic strength (Matsuda & Podolsky, 1984; Brenner *et al.*, 1984).

The existence of a series elastic element associated with each crossbridge which can extend by about 40 Å under maximum load obviously fits in with a 40 Å active working stroke of the contractile elements in a straightforward way. However, it may at first appear paradoxical that such a system would give rise to the quick release–early rapid recovery phenomena over much longer release distances (i.e. up to 120 Å) as seen by Huxley & Simmons (1971). Nevertheless, the effect could arise in a simple way if there were always many weakly bound crossbridges in the region on actin from say $x = 40$ Å to 120 Å (Figs 1 and 2) and if a proportion of these could undergo a rapid transition to the strongly bound, tension generating state when they were translated into the 0–40 Å region on actin. In this model, it is being assumed (i) that initial binding is to the weak state and takes place predominantly in the region 40 Å to 120 Å (the 'waiting zone'), (ii) that a rate-limiting step has to take place either in this weak binding state, or prior to binding (Stein *et al.*, 1984) before such a bridge is competent to develop tension when it is moved subsequently to the 'active zone' (0–40 Å) and (iii) that a crossbridge ceases to develop tension and rapidly detaches when it is conveyed to negative values of x. Thus a rapid release of 40 Å from an isometric contraction would discharge all the pre-existing tension-generating crossbridges, but at the same time bring a new population of crossbridges into the active zone. Since these had already 'equilibrated' in the 'waiting zone', they would contain the normal proportion of competent bridges, and rapid redevelopment of a high level of tension would occur. The distribution of attached states between $x = 40$ Å and $x = 120$ Å would determine the shape of the T_2 curve. This type of explanation of the rapid tension recovery effect has some similarities to, but also some important differences from, that described by Eisenberg & Hill (1978). It differs

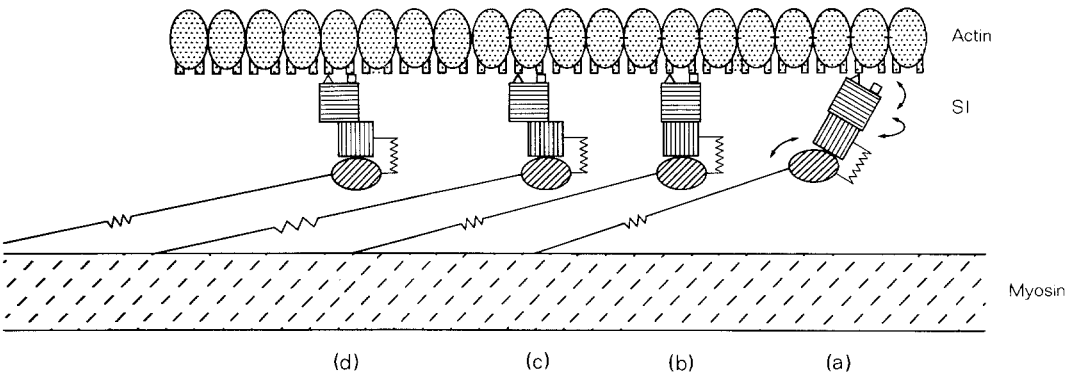

Fig. 1. Schematic diagram illustrating different configurations of myosin crossbridges during the part of the crossbridge cycle when they are attached to actin. (a) represents the initial weakly attached state (possibly one-site binding) in which it is supposed that the S1 head can adapt a variety of orientations relative to the actin filament and may also have considerable internal flexibility. (b) represents a short-lived configuration in which the transition to strong attachment (possibly two-site binding) can first take place. This quickly triggers a structural change in the S1 head which extends the elastic element (possibly but not necessarily in S2) by about 40 Å so that the crossbridge goes over into configuration (c). (c) represents the configuration of the tension-generating crossbridges, in which the form of attachment of the S1 head is close to, but not identical to, that in the rigor state. The elastic part of the crossbridge (shown here diagrammatically as an extensible element in S2) has been stretched and is generating tension and shortening as the actin and myosin filaments slide past each other. (d) represents the configuration at the end of the working stroke. The elastic element has now shortened back to its equilibrium length and is no longer generating tension. The S1 head can now adopt the normal rigor-like attachment (perhaps by dissociation of ADP) and can be detached from actin following the binding of the next molecule of ATP. (For simplicity, only single myosin heads shown.)

from the Huxley–Simmons type of explanation in that it supposes that rapid tension recovery involves the activation of a new set of crossbridges rather than transitions between multiple attached states of crossbridges which were already generating tension before the quick release.

This type of model has the advantage that the structural change in the crossbridge has only to produce 40 Å of active axial movement, instead of the 120 Å which has often been assumed to be required. It would therefore be much easier to accommodate this change within what might appear to be a reasonable range of movement even if only part of the S1 structure were involved. Such restricted amounts of movement would be correspondingly easier to accommodate with the results of the probe studies. Moreover, in such a mechanism it would be possible for the contractile element to go over immediately to almost its final state, stretching the elastic element fully, once the critical relative positions of the actin and myosin units involved had become accessible (e.g. at $x = 40$ Å) (see Fig. 2). Subsequent shortening would then involve only a return of the elastic elements towards their initial length, and all the tightly bound heads would always be in virtually the same configuration. This would have the advantage of

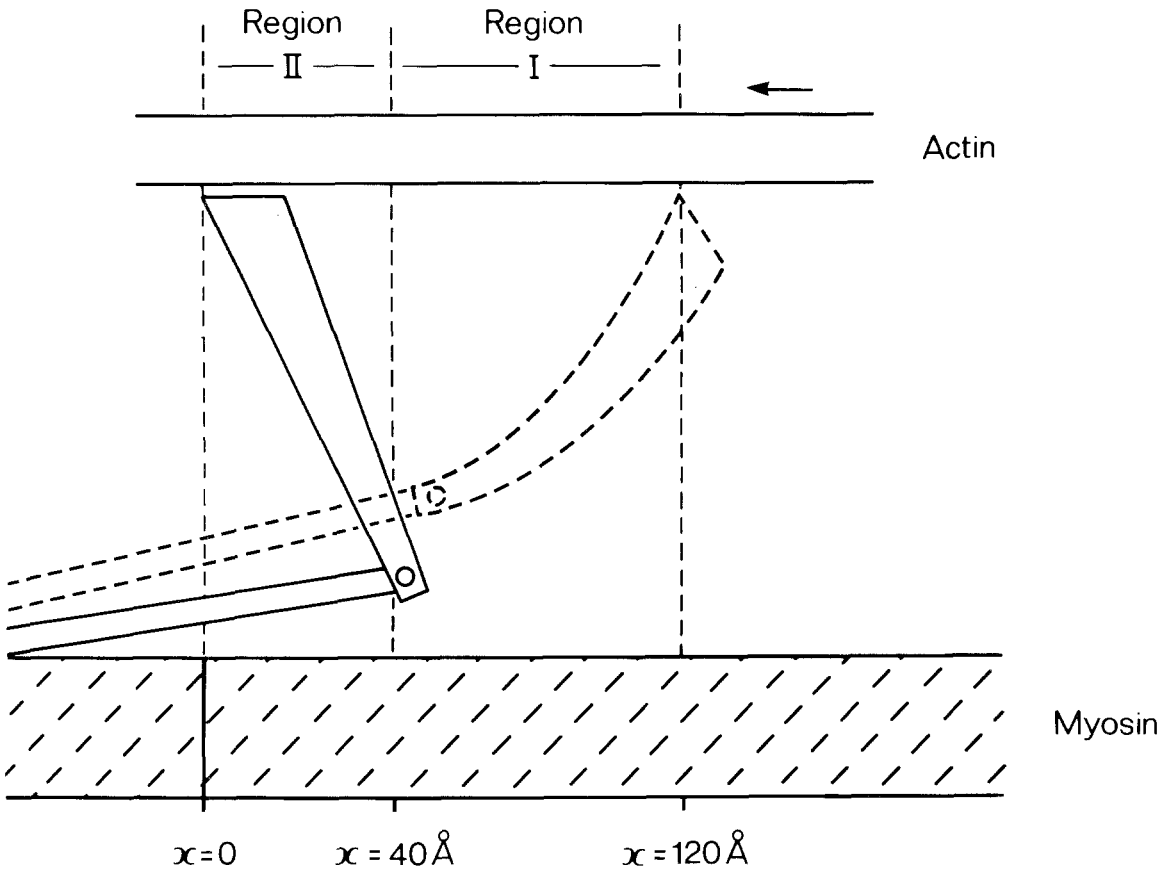

Fig. 2. Diagrammatic representation of the two regions on an actin filament which are specified by the axial position of a myosin molecule which is forming a crossbridge. The position $x = 0$ corresponds to the end of the working stroke with the elastic element in the crossbridge at its equilibrium length, the S1 head forming a rigor-like attachment to actin, and no tension being generated. The region between $x = 0$ and $x \sim 40$ Å (Region II) represents positions where strongly bound, tension-generating crossbridges can be attached to actin. The region between $x \sim 40$ Å and $x \sim 120$ Å (Region I) represents positions where strong binding does not occur. Initial attachment of crossbridges is to the weakly bound state and takes place mainly in Region I, but there is a finite probability of such initial attachment in Region II. In a shortening muscle, where a crossbridge has attached initially in Region I, the transition to the strongly bound, tension-generating state can take place very rapidly as soon as the attachment site crosses the boundary from Region I to Region II. However, a rate-limiting step is present in the system which controls the proportion (at different speeds of shortening) of myosin heads in Region I that are competent to generate tension when they reach Region II. In a quick release, the initial tension drop occurs when some or all of the crossbridges in the original Region II are brought to or beyond the end of their working stroke. Rapid tension recovery occurs when the population of competent crossbridges, which have now been moved from a Region I to a Region II position, rapidly go over into a strongly bound, tension-generating state. (The crossbridges have been deliberately drawn in an extremely schematic and unrealistic manner, in order to make the positional relationships clearer.)

allowing the chemical reaction associated with energy transduction to take place in an all-or-none fashion, and would also account for the probe results which detect only a rigor like configuration. Presumably, the actual configuration would be slightly different from rigor while tension was still being applied to it, so that dissociation of ADP would be delayed until the appropriate point in the cycle.

These tension generating crossbridges, which would repeat axially within ± 20 Å of a 143 Å periodicity, could contribute to the 143 Å axial reflection seen in contracting muscle, and the change in the intensity of that reflection with tension at different speeds of shortening could be a consequence, in part, of a change in the number of crossbridges in the tension generating state.

A possible implication of this type of model is that the two different attached states of the crossbridge, at the beginning and end of the working stroke, are in fact the two states we have already identified, one of them similar (though probably not identical) to rigor, the other a loosely attached state perhaps involving a flexible and internally mobile form of the myosin head. Thus instead of searching for two different static configurations, one might attempt to characterize more clearly the dynamic state of the weakly attached crossbridges.

References

BRENNER, B., SCHOENBERG, M., CHALOVICH, J. M., GREEN, L. E. & EISENBERG, E. (1982) Evidence for cross-bridge attachment in relaxed muscle at low ionic strength. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7288–91.

BRENNER, B., YU, L. C. & PODOLSKY, R. J. (1984) X-ray diffraction evidence for crossbridge formation in relaxed muscle fibres at various ionic strengths. *Biophys. J.* **46**, 299–306.

BURGHARDT, T. P., ANDO, T. & BOREJDO, J. (1983) Evidence of cross-bridge order in contraction of glycerinated skeletal muscle. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7515–19.

CHALOVICH, J. M. & EISENBERG, E. (1982) Inhibition of actomyosin ATPase activity by troponin–tropomyosin without blocking the binding of myosin to actin. *J. biol. Chem.* **257**, 2432–7.

COOKE, R. & FRANKS, K. E. (1980) All myosin heads form bonds with actin in rigor rabbit skeletal muscle. *Biochem. J.* **19**, 2265–9.

COOKE, R., CROWDER, M. S. & THOMAS, D. D. (1982) Orientation of spin labels attached to cross-bridges in contracting muscle fibres. *Nature, Lond.* **300**, 776–8.

COOKE, R., CROWDER, M. S., WENDT, C. H., BARNETT, V. A. & THOMAS, D. D. (1984) Muscle cross-bridges: do they rotate? In *Contractile Mechanisms in Muscle* (edited by POLLACK, G. H. and SUGI, H.), pp. 413–23. New York, London: Plenum Press.

EISENBERG, E. & HILL, T. L. (1978) A crossbridge model of muscle contraction. *Prog. biophys. molec. Biol.* **33**, 55–82.

FORD, L. E., HUXLEY, A. F. & SIMMONS, R. M. (1977) Tension responses to sudden length change in stimulated frog muscle fibres near slack length. *J. Physiol., Lond.* **269**, 441–515.

FORD, L. E., HUXLEY, A. F. & SIMMONS, R. M. (1981) The relation between stiffness and filament overlap in stimulated frog muscle fibres. *J. Physiol., Lond.* **311**, 219–49.

HASELGROVE, J. C. & HUXLEY, H. E. (1973) X-ray evidence for radial cross-bridge movement and for the sliding filament model in actively contracting skeletal muscle. *J. molec. Biol.* **77**, 549–68.

HUXLEY, A. F. & SIMMONS, R. M. (1971) Proposed mechanism of force generation in striated muscle. *Nature, Lond.* **133,** 533–8.

HUXLEY, H. E. (1975) The structural basis of contraction and regulation in skeletal muscle. *Acta anatomica nipponica* **50,** 310–25.

HUXLEY, H. E. (1979) Time resolved X-ray diffraction studies on muscle. In *Crossbridge Mechanism in Muscle Contraction* (edited by SUGI, H. and POLLACK, G. H.), pp. 391–405. Tokyo: University of Tokyo Press.

HUXLEY, H. E. (1980) The movement of myosin cross-bridges during contraction. In *Muscle Contraction: its Regulatory Mechanisms* (edited by EBASHI, S., MARUYAMA, K. and ENDO, M.), pp. 33–43. Tokyo: Japan Science Society Press, and Berlin: Springer-Verlag.

HUXLEY, H. E., FARUQI, A. R., KRESS, M., BORDAS, J. & KOCH, M. H. J. (1982) Time-resolved X-ray diffraction studies of the myosin layer-line reflections during muscle contraction. *J. molec. Biol.* **158,** 637–84.

HUXLEY, H. E., SIMMONS, R. M., FARUQI, A. R., KRESS, M., BORDAS, J. & KOCH, M. H. J. (1983) Changes in the X-ray reflections from contracting muscle during rapid mechanical transients and their structural implications. *J. molec. Biol.* **169,** 469–506.

HUXLEY, H. E., KRESS, M. & FARUQI, A. R. (1984). Time resolved X-ray diffraction measurements of the activation mechanism in striated muscle. *Biophys. J.* **45,** 10a.

HUXLEY, H. E., KRESS, M. & SIMMONS, R. M. (1985) Time resolved X-ray diffraction studies of muscle activation and contraction. *Biophys. J.* **47,** 24a.

KUSHMERICK, M. J. & DAVIES, R. E. (1969) The chemical energetics of muscle contraction. *Proc. R. Soc.* Ser. B. **174,** 315–53.

LOWELL, S. J. & HARRINGTON, W. F. (1981) Measurement of the fraction of myosin head bound to actin in rabbit skeletal myofibrils in rigor. *J. molec. Biol.* **149,** 659–74.

MATSUBARA, I., YAGI, N., MIURA, H., OZEKI, M. & IZUMI, T. (1984) Intensification of the 5.9 mm actin layer line in contracting muscle. *Nature, Lond.* **312,** 417–73.

MATSUDA, T. & PODOLSKY, R. J. (1984) X-ray evidence for two structural states of the actomyosin cross-bridge in muscle fibers. *Proc. natn. Acad. Sci. U.S.A.* **81,** 2364–8.

PODOLSKY, R. J., ST. ONGE, R., YU, L. & LYMN, R. W. (1976) X-ray diffraction of actively shortening muscle. *Proc. natn. Acad. Sci. U.S.A.* **73,** 813–17.

STEIN, L. A., CHOCK, P. B. & EISENBERG, E. (1984) The rate-limiting step in the actomyosin adenosine triphosphatase cycle. *Biochem.* **23,** 1555–63.

THOMAS, D. D. & COOKE, R. (1980) Orientation of spin-labeled myosin heads in glycerinated muscle fibers. *Biophys. J.* **32,** 891–906.

YAGI, N., ITO, M. H., NAKAJIMA, H., IZUMI, T. & MATSUBARA, I. (1977) Return of myosin heads to thick filaments after muscle contraction. *Science* **197,** 685–7.

YANAGIDA, T. (1981) Angles of nucleotides bound to cross-bridges in glycerinated muscle fibre at various concentrations of εATP, εADP, and εAMPPNP detected by polarized fluorescence. *J. molec Biol.* **146,** 539–60.