Biophysical Chemistry, 32 (1988) 111–119
Elsevier

PBC 01317

Evaluation of mechanical parameters of the mechanochemically 'active state' of actin filaments on the basis of purely chemical data

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Received 13 April 1988
Revised manuscript received 27 July 1988
Accepted 1 August 1988

Actin; ATPase; DNase 1; Heavy meromyosin; Mechanochemistry

Oosawa and his collaborators (cf. F. Oosawa, Biophys. Chem. 11 (1980) 443), employing various optical techniques, have shown that the flexibility of actin filaments increases upon interacting with the enzymatically active myosin fragments, particularly heavy meromyosin (HMM). It has been reported (S. Hitchcock, L. Carlsson and U. Lindberg, Cell 7 (1976) 53) that HMM can accelerate the DNase 1-induced depolymerization of F-actin, provided MgATP is also present. Since, as we have demonstrated (cf. J. Borejdo and A. Oplatka, Biochim. Biophys. Acta 440 (1976) 241), HMM, like filamentous myosin, is endowed with mechanochemical capability, we made an attempt to correlate the enhanced rate of depolymerization with the decrease in rigidity of the G–G bonds in F-actin. On the basis of the chemical kinetic data of Hitchcock et al. we could derive the approximate value of the HMM-MgATP-induced change in rigidity which is a mechanical molecular parameter. Since interaction between HMM or HMM subfragment-1 and F-actin in the presence of MgATP leads to the movement of the myosin heads along the actin filaments, it is argued that the enzymic behavior of this system should not be analyzed on the basis of simple, equilibrium, complex formation.

1. Introduction

Upon stimulation, muscle undergoes profound changes which are regarded as the outcome of a change in state – from the relaxed to the active state. The marked increase in stiffness and the contractile force developed are ascribed to an interaction between the myosin heads and the surrounding actin filaments. Unfortunately, most of the efforts invested so far in the attempt to elucidate the mechanism of muscular contraction have been concerned with conformational changes in the myosin molecules, neglecting the possibility that their equal partner, namely the actin filaments, may also experience changes which could in principle affect their thermodynamic parameters and in particular their stability and affinity to other protein components. Oosawa and his collaborators [1–5] have been practically the only ones who persistently stressed this idea and presented data, obtained by a variety of techniques, indicating that the flexibility of actin filaments changes upon interaction with myosin derivatives, in both the presence and absence of MgATP, Ca^{2+}, and the regulatory proteins, troponin and tropomyosin. Most relevant to the active state of muscle were experiments in which the enzymatically and mechanochemically [6,7] active double-headed myosin fragment heavy meromyosin (HMM) was added together with MgATP to F-actin (or to F-actin in the presence of the regulatory proteins and Ca^{2+}). Under these conditions, the values of the elastic modulii for bending and for stretching of the actin filaments are smaller by a factor of about two as compared to the values for the free filaments, i.e., in the absence of HMM. The microscopic elastic constant of a G-

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actin–G-actin bond in a filament should also decrease by the same factor [2].

In a study of the kinetics of DNase 1-indueed depolymerization of actin filaments [8,9], Hitchcock et al. observed that, while depolymerization was inhibited when saturating amounts of HMM or of its subfragment-1 (S-1) were present, the addition of both HMM (or S-1) and MgATP caused a much faster depolymerization, as demonstrated by faster drops in viscosity and in DNase 1 activity. Since MgATP dissociates the acto-HMM complex, these authors suggested that when myosin heads interact with the filaments, the actin subunits go through some kind of conformational change which persists after release of HMM and either makes the available sites bind DNase 1 more tightly or increases the number of binding sites per filament, and this would then lead to a more rapid depolymerization.

As indicated by Hitchcock et al. [9] the major factor operating during the depolymerization of F-actin by DNase 1 does not seem to be the tight binding of the enzyme to monomeric G-actin leading to depolymerization in an effort to restore the original $F \rightleftarrows G$ equilibrium. Thus, profilin, just like DNase 1, prevents the polymerization of G-actin, but prolonged incubation of F-actin with profilin does not result in its breakdown [10]. Several findings [9] lend support to the idea that DNase 1 binds to F-actin thus somehow weakening G–G bonds, enabling a relatively fast release of actin monomers which are then bound irreversibly to DNase 1 molecules.

The increase induced by HMM plus MgATP in the rate of DNase 1-induced depolymerization could suggest further weakening of the G–G bonds. If indeed, in the presence of MgATP, HMM can destabilize F-actin, then one could expect that, in the absence of DNase 1, the interaction between HMM, MgATP and F-actin should lead to a shift in the $F \rightleftarrows G$ equilibrium in favor of G-actin. This would indicate a rise in the chemical potential of the actin subunits in F-actin. However, it has been reported by Tawada and Oosawa [11] that HMM in the presence of MgATP produces no change in the critical concentration and accelerates both polymerization and depolymerization to the same extent, whereas in the absence of MgATP it causes a shift in favor of F-actin. As stated by these authors, such a behaviour is expected if HMM-MgATP "has no strong affinity for either G- or F-actin, but has a specific affinity for an intermediate or transient state between G-actin and F-actin or has the ability to bring actin to that intermediate state". In other words: when HMM and MgATP are added to actin, the chemical potential of neither F-actin nor G-actin is affected. The induced increase in the rate of depolymerization indicates that the value of the activation free energy is decreased, suggesting that the value of the chemical potential of the 'activated complex' is lowered.

One should, however, bear in mind the basic fact that the interaction between HMM or S-1 and F-actin in the presence of MgATP involves the enzymic splitting of the nucleotide. Moreover, HMM and S-1, just like filamentous myosin, have mechanochemical capability so that the enzymatic reaction can be accompanied by the translocation of the actin filaments and, under appropriate conditions, tension generation [6,7,12]. This has been verified by Tawada et al. [13], Yano, Shimizu and coworkers [14,15], Yanagida et al. [16,17] and Spudich et al. [18], in both in vivo and in vitro systems. Both the changes in the values of the elastic modulii discussed above and the 'activation' of depolymerization in the presence of DNase 1 might well be due to the mechanochemical reactivity and thus related to each other.

In the following, we will consider the possibility that the decrease in the value of the elastic constant of the G–G bond, due to interaction with myosin heads, is responsible for the anticipated decrease in the activation free energy, leading to the observed increase in the value of the dissociation constant in either or both ends of the actin filaments. As in the case of pure F-actin, the more rapidly released monomers will be 'caught' by DNase 1 molecules, thus prohibiting their reassociation. This will obviously lead to an increase in the rate at which DNase 1 molecules are deactivated. The experimentally observed increase will be related quantitatively to the decrease in the value of the elastic constant of the G–G bond associated with the mechanochemically 'active state' of the actin filaments.

2. The mechanical component of the activation energy of the depolymerization process

The activation free energy of a chemical reaction is the value of the 'energy barrier' which should be overcome for the reaction to proceed. In the case of the dissociation of an actin monomer from either end of an actin filament this consists, at least partly, of the work required for stretching the G–G bond to a point at which the chemical potential of the 'active complex' can drop abruptly and irreversibly thus leading to the 'ejection' of a monomer into the medium, i.e., to dissociation. We may, therefore, write the dissociation constant q as

$$q = q_0 \cdot e^{-\int f \, dl/kT} \tag{1}$$

where f is the stretching force and l the length of the bond. The integration limits are the 'resting' bond length and the length at which dissociation occurs. However, assuming that f changes linearly with l, i.e., that $\Delta f/\Delta l$, the stretching constant of the G–G bond, is indeed constant,

$$\int f \, dl = \int (\Delta f/\Delta l) \cdot \Delta l \, dl = 1/2(\Delta f/\Delta l) \cdot (\Delta l_0)^2 \tag{2}$$

where Δl_0 is the stretching distance at dissociation. Hence

$$\ln q = -(1/2kT) \cdot (\Delta f/\Delta l) \cdot (\Delta l_0)^2 + \ln q_0 \tag{3}$$

The change in q following activation by HMM + MgATP is thus given by

$$\Delta \ln q = -(\Delta l_0)^2/2kT \cdot \Delta(\Delta f/\Delta l) \tag{4}$$

According to Oosawa et al. [4], taking the radius d of G-actin as the radius of a single strand of F-actin,

$$\Delta f/\Delta l = 2\epsilon/d^3 \tag{5}$$

where ϵ is the flexural rigidity. The value of ϵ has been estimated to be 6.5×10^{-17} dyn cm^2 for a free actin filament whereas in the presence of HMM and MgATP it is equal to 3.5×10^{-17} dyn cm^2 [5]. Assuming that HMM plus MgATP weaken the G–G bonds to the same extent in the presence and absence of DNase 1, i.e., that the change of $\Delta f/\Delta l$ in the activation free energy is the same in both cases, and taking d to be 27.5 Å [19], we obtain from eq. 4

$$\Delta \ln q = -(1/d^3kT) \cdot \Delta l_0^2 \Delta\epsilon = 3.6 \times 10^{16} (\Delta l_0)^2 \tag{6}$$

Analysis of the data presented by Hitchcock et al. [9] shows that the initial rate of drop in the inhibition of DNase 1 due to depolymerization of F-actin becomes 5-times larger when HMM and MgATP are added under similar conditions. This corresponds to $\Delta \ln q$ of 1.6. Hence, from eq. 6, $\Delta l_0 = 0.67$ Å.

We may reverse the argument and, taking Δl_0 to be 1 Å (which is the order of the breaking of a chemical bond), we can, on the basis of experimental data such as those obtained by Hitchcock et al. [9], obtain at least a rough estimate of the changes in the flexural rigidity and in the bond elastic constant probably resulting from mechanochemical activity. In other words: our analysis, which utilizes purely chemical data, can provide us with an approximate value for a mechanical parameter.

The free energy increase associated with the stretching of the G–G bond in a 'relaxed' free actin filament is, from eqs. 1, 2 and 5 and with the above mentioned value of $\epsilon = 6.5 \times 10^{-17}$, equal to:

$$\int f \, dl = 1/2(\Delta f/\Delta l)\Delta l_0^2 = (\epsilon/d^3) \cdot \Delta l_0^2$$
$$= 1.4 \times 10^{-13} \text{erg} = 2.05 \text{ kcal/mol}.$$

This value is of the same order as the free energy gain during the polymerization of actin as previously shown [20].

So far, we have described the depolymerization of actin by a single dissociation constant, as if dissociation occurred at only one end of the filaments, i.e., at the barbed end or at the pointed tip. However, the same treatment should also be valid for dissociation occurring at both ends, even if the value of the rate constant for dissociation differs for the two ends, since the concentration term in the kinetic equation should be the same and the dissociation constant q (eq. 1) would then represent the sum of the different q values. It is

interesting to note that, while the association rate constant is 5–10-times smaller at the pointed, compared to the barbed, end of the actin filaments, the dissociation rate constants at both ends are the same and assume the value of 1–2 s^{-1} [21].

Upon introducing the values obtained above for the activation free energy changes associated with stretching into eq. 1, and taking into consideration that $q = 1–2\ s^{-1}$, we can get an idea of the relative contributions of the mechanical and the 'non-mechanical' terms to the value of the dissociation constant. For the relaxed actin filaments at 25 °C:

$$q = 1.5 = q_0 \cdot e^{-2050/RT} = q_0 \cdot 0.0308\ s^{-1}$$

Hence, $q_0 = 48.7\ s^{-1}$ or $q = 48.7 \times 0.0308\ s^{-1}$ where the first and the second terms are, respectively, the non-mechanical and mechanical terms. On the other hand, for the activated filament, for which q is 5-times larger,

$$q = 5 \times 1.5 = 48.7(5 \times 0.0308) = 48.7 \times 0.154\ s^{-1}$$

The mechanical term

$$\exp - \left[(1/2kT) \cdot (\Delta f/\Delta l) \cdot (\Delta l_0)^2\right]$$

$$= \exp - \left[(1/2kT) \cdot [\Delta f_0/(\Delta l_0)] \cdot (\Delta l_0)^2\right]$$

$$= \exp - \left[(1/2kT) \cdot \Delta f_0 \cdot (\Delta l_0)\right],$$

(where Δf_0 is the value of f at Δl_0) is analogous to the term $\exp - [P\Delta V^{\pm}/RT]$ which represents the effect of applied pressure P on the value of the rate constant of a chemical reaction in which the volume (per mole) of the activated complex differs by $\Delta V^{\pm}$ from the combined volumes of the reactants.

It is interesting to note that analysis of the experiments reported by Tawada and Oosawa [11] indicates that in low ionic strength medium, HMM plus MgATP accelerate the rate of depolymerization of F-actin by a factor not much different from the value of 5 obtained from the DNase 1-induced depolymerization experiments, [8,9] (which were carried out in the presence of 0.1 M NaCl) for similar concentrations of the proteins (data not shown).

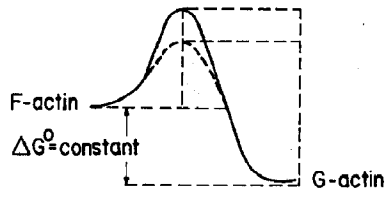

Fig. 1. Lowering of the activation energies of the depolymerization and the polymerization reactions of actin to the same extent without affecting the value of the standard free energy change ΔG^0 (- - - - -) Active complex formation in the presence of HMM and MgATP. (———) Same for F-actin alone.

Since, as mentioned above, the critical concentration of actin is not affected by HMM in the presence of MgATP, it is clear that the rate constant for polymerization must be elevated by the same factor as the dissociation constant. Evidently, this factor is associated with the change induced in the filamentous actin by the mechanochemical activation, the actin monomers being unaffected. The increase in the flexibility of actin filaments thus also accelerates the association of actin monomers. Fig. 1 shows that actually it has to be anticipated that any change in the value of the activation energy of a reaction, due only to a modification of the chemical potential of the activated complex, should give rise to exactly the same change in the value of the activation energy of the reverse reaction.

In summary, the mechanochemical interaction between F-actin and myosin heads does not affect the chemical potential of the actin filaments despite the fact that concurrently the value of the elastic constant of the G–G bonds is decreased, leading to lowering of the energy barriers of both polymerization and depolymerization reactions. At first sight, this is quite astonishing since, after all, the filaments have undergone what seems to be a profound change. A solution of this riddle could be that a change in the value of the chemical potential should be anticipated only if an external force is applied longitudinally, stretching the filaments which became more flexible by the interaction with HMM, thus 'investing energy' in the G–G bonds and elevating the chemical potential. This does not happen with F-actin filaments in solution and the change in flexibility is exhibited only as an easier transition to the activated com-

plex. In an isometrically contracting muscle, on the other hand, an external mechanical force is operating, the direction of which is opposite to that of the contractile force, thus keeping the length constant. The chemical potential should then be higher than in the relaxed muscle. The driving force for the depolymerization of the thin filaments should, therefore, become larger upon stimulation if muscle length is held constant. Apparently, the attachment of the filaments to the Z-bands and the capping of the pointed ends by β-actinin [22] protect against such destabilization, if a continuous three-dimensional protein network exists in isometrically contracting muscles.

Since, as indicated above, the rate constants for association differ for the pointed and the barbed ends of the filaments by a factor of 5–10 and as, in the presence of MgATP, the critical concentration of actin is 12–15-fold higher at the pointed than at the barbed end [21], we may conclude that each of the two rate constants for association is increased by the same factor if we assume that the critical concentrations at the two ends are not influenced by the interaction with HMM.

At equilibrium, in the presence of MgATP, actin filaments grow at the barbed end – and undergo net depolymerization at the same rate at the pointed end – so that the length of the filaments remains constant [23]. Polymerization is followed by the splitting of MgATP, originally bound to the attached G-actin monomers, which thus occurs near the ends of the filaments [24]. Acceleration to the same extent of both polymerization and depolymerization by HMM in the presence of MgATP means that treadmilling becomes faster as a consequence of mechanochemical reactivity. Treadmilling will cause the advancement of a filament (which continuously changes its constituent monomers) in a non-muscle cell in the direction of the barbed end, and this is paid for by the hydrolysis of MgATP [23]. This translocation thus occurs in a direction which is opposite to the direction of movement of the actin filaments in an active striated muscle, in which contraction occurs in the direction of the pointed ends [25]. Thus, myosin may have a dual effect on the localization of actin filaments in non-muscle cells: it may induce translational movement of the filaments in the direction of their pointed end while, at the same time, it may accelerate growth at the barbed ends at the expense of the pointed ends. If the rates of the two opposite movements are equal, there will be no net movement in either direction. This is analogous to a person running in a train in a direction opposite to its direction of travel and at the same velocity. An outside observer will not detect any movement of that person relative to the ground. As we have seen, HMM may cause an increase in the rate of depolymerization at the pointed ends by a factor of five at pH and ionic strength values quite close to physiological. This must also be the increase in the rate of polymerization. As the value of the rate constant for the depolymerization of free F-actin is (as indicated above) about 1.5 s^{-1}, the rate of polymerization should be $5 \times 1.5 = 7.5$ s^{-1} or 7.5 monomers added per s (i.e., 3.75 monomers per strand of the double-stranded actin filament). With 55 Å as the diameter of an actin monomer [19], we obtain 206 Å s^{-1} for the velocity of translocation of the barbed end due to growth. An F-actin filament which is blocked at its ends by capping proteins will move as a whole in the opposite direction when MgATP is split at the site of interaction between the myosin heads and F-actin. This velocity depends on the origin of the myosin component and could vary in muscles from 0.1 to 24 μm per s, proportionally to the specific actin-activated ATPase activity of the myosin [26]. If this velocity is of the order of 200 Å s^{-1} and if the ends of the filaments are not capped, then the opposite movements will cancel each other and no net movement will take place. This may happen, in principle, in non-muscle cells. Translocation of actin filaments also does not occur in an isometrically contracting muscle. In this system, however, interaction between myosin heads and the accompanying splitting of MgATP take place while no treadmilling occurs at the ends which are both capped [22].

3. The splitting of MgATP by acto-HMM (or acto-S-1 or actomyosin) is not a simple 'Michaelis-Menten' enzymic reaction because the myosin heads are continuously on the move along the actin filaments

As stated above, the fact that the chemical potential of F-actin is not affected by its mechanochemical interaction with HMM suggests that the affinity of the myosin heads to F-actin is negligible. On the other hand, when MgATP is absent, strong 'rigor' bonds form between the two proteins and this leads to (a) a decrease in the critical concentration of actin (suggesting a decrease in the value of the chemical potential of the actin subunits in F-actin) and (b) full inhibition of depolymerization by DNase 1 (indicating strong binding of the myosin heads to actin). The low affinity in the presence of MgATP is in line with the conclusion derived by Eisenberg and his associates [27], on the basis of viscosity, turbidity, and laser-light fluctuation autocorrelation measurements of acto-HMM and acto-S-1 solutions, that practically all of the myosin heads are dissociated from actin, even under conditions where the actin-activated Mg^{2+}-ATPase activity is close to its maximal value under the ionic strength conditions employed in the DNase 1-induced depolymerization experiments [9], i.e., in the presence of 0.1 M NaCl. This conclusion is based on the idea that complexes are formed between myosin-MgATP heads and actin, in which a given myosin head is bound to the same actin subunit (throughout the Mg^{2+}-ATPase cycle), just as is the case for rigor complexes. The kinetics of the Mg^{2+}-ATPase activity of acto-HMM (or S-1) have also been interpreted with this picture in mind and were characterized by a Michaelis-Menten constant [28,29]. However, this view cannot hold if we take into consideration the more recent finding that HMM, like filamentous myosin, is mechanochemically, and not only enzymatically, active and is 'entitled' to 'slide' along actin filaments when MgATP is present, just like filamentous myosin. Thus, Yanagida et al. [16] could observe the translocation of fluorescently labeled HMM towards the Z-bands in skeletal muscle myofibrils from which myosin had been extracted ('ghosts') when MgATP was added. An HMM-MgATP molecule which interacts with F-actin thus does not form a definite complex with one or two actin subunits, with the option of either back-dissociating or, with the help of the bound actin subunit(s), hydrolyzing the MgATP molecule and then dissociating upon the arrival of a fresh MgATP molecule. Rather, upon 'contacting' an actin filament, a myosin head might very soon start 'rolling' along the filament towards its barbed end. According to Yanagida et al. [30], the sliding distance, i.e., the distance covered by a myosin head along an actin filament which is associated with the breakdown of a single ATP molecule, is 700–1000 Å. A rolling myosin head thus 'interacts' with $700 : 55.5 = 14$ or more actin subunits so that there is no such entity that resembles a 'rigor complex' in terms of stoichiometric complex formation. The evluation of the percentage of myosin heads which are complexed with actin subunits thus becomes more complicated. The fact that, on the contrary, the number of HMM or S-1 molecules which co-sediment with actin in an ultracentrifuge is small, compared with the number anticipated, indicates that the number of heads rolling at any given moment along an actin filament is also relatively small. An HMM (or S-1) molecule moving along an actin filament will necessarily eventually arrive at the barbed end of that filament and will then probably be 'shot' into the surrounding fluid, away from the filament in the same direction.

In summary, the interaction between HMM (or S-1 or myosin) and F-actin in the presence of mgATP cannot and should not be considered as a random interaction obeying the mass action law as is the case with other enzymic reactions which take place between one or more globular proteins and a low-molecular-weight substrate. The reaction is vectorial and, moreover, results in a directional translocation of the proteins relative to each other, thus enabling the myosin heads to arrive at another segment of the actin filament without having to 'search' for it by moving randomly in the medium (as is the case for a globular enzyme molecule in solution).

The movement of the myosin heads along a pre-determined path in a unique direction, which

is associated with the accompanying directional active streaming of water [12], could affect both the enzymic activity and the values of experimental parameters that have been used for the determination of the extent of 'binding' of HMM or S-1 to actin in the presence of MgATP. Thus, the experiments performed by Eisenberg et al. [27] at extremely low ionic strength have led to two different sets of values for the percentage of HMM or S-1 molecules bound to actin under conditions close to maximal activation of the Mg^{2+}-ATPase activity (e.g., saturation of HMM with the highest possible excess of actin): while viscosity, turbidity and laser-light fluctuation autocorrelations studies gave values of the order of 2%, ultracentrifugation experiments indicated that about 40 and 20%, respectively, of the HMM and the S-1 molecules, were bound, i.e., an order of magnitude larger. Viscosity and turbidity were analyzed as follows: the viscosity of actin-HMM mixtures at low ionic strength in the presence of MgATP was compared with that measured when all of the ATP present was hydrolyzed (using very low ratios of HMM to actin). It was found that when all the ATP was depleted it took only 2% as much HMM to cause the same viscosity change in the absence of MgATP as it took in its presence. Therefore, assuming that viscosity was a linear measure of actin-HMM binding, the data suggested that only about 2% of the HMM is bound to actin under conditions of maximal actin activation. Similarly, assuming that turbidity approximates a linear measure of the binding between the myosin subunits and actin, the data suggested that very little binding of HMM or S-1 to actin occurs under these conditions. In other words, the turbidities of acto-HMM and of acto-S-1 were found to be practically identical to the sum of those of the separate proteins.

These authors realized that the assumption that viscosity and turbidity are linear measures of binding between actin and the myosin fragment might not be quantitatively accurate. Furthermore, they raised the question of whether it was possible "that viscosity and turbidity are not a measure of binding between actin and HMM or S-1 at all and in fact complete binding can occur in the presence of MgATP without any increase in viscosity and turbidity. If this were true, it would mean that unlike acto-HMM which has a very high viscosity and turbidity, acto-HMM-MgATP would have the same low viscosity and turbidity as dissociated actin and HMM". The very fact that in ultracentrifugation experiments actin filaments still appear to be capable of binding up to 20% of what has been thought to be their full capacity for binding S-1 should mean that the value of 2% derived from the viscosity and turbidity measurements (which were carried out employing the same solutions) may hide some effects which could not be considered at that time. The discovery [6,7,12–18] that HMM and S-1 are endowed with mechanochemical, and not only enzymic, activity suggests that the active streaming along actin filaments, which is intimately associated with mechanochemical activity of the soluble myosin fragments [12], should be considered when analyzing the viscous forces operating on an actin filament in viscosity measurements. The force linked with active streaming apparently acts in a direction which is opposite to the viscous force due to the relative movement of the actin filaments and their surrounding fluid which occurs as a result of the flow of the fluid in the viscometer. Since the direction of active streaming is related to the polarity of the filaments, and as streaming appears to proceed from the barbed towards the pointed ends [31], we must conclude that the filaments in the viscometer are to some (or to a large) extent oriented in the direction of flow so that the front end is the pointed one. Orientation of the filaments parallel to each other should be facilitated by the individual flows along each of the filaments [32]. Bundles, each consisting of several parallel actin filaments, could indeed be observed in the electron microscope when HMM was added to F-actin, provided MgATP was also present. However, if under the same conditions and at the same protein concentrations, ATP was totally excluded by mixing HMM with F-actin from which ATP had been removed by dialysis, an isotropic network consisting of single actin filaments was observed [33]. Bundle formation may also cause further decrease in the viscosity. Parallel orientation of the active filaments may also lead to a decrease in light scattering, possibly

down to the level of the practically fully separated myosin fragments and actin.

In summary: the degree of binding of HMM or S-1 to actin in the absence of added NaCl or KCl is probably truly represented by the ultracentrifugation experiments which means that, at any moment, up to 20% (in the case of S-1) and 40% (for HMM) are 'associated' to actin (instead of the 85% expected when the Mg^{2+}-ATPase activity amounts to 85% of V_{max} [27]. Since this association does not reflect real binding (as in the case of rigor complex formation) but rather the close presence of fast moving myosin heads, there is no reason why dissociation of actin monomers from the filaments' ends should be hindered (as it is in the absence of MgATP) to any extent.

Eisenberg et al. have proposed that, at any time, a large fraction of the HMM or S-1 molecules is in a refractory state unable to bind to actin, while only a small fraction is in a non-refractory state which can form a complex with actin [27]. In view of the above, we may conclude that the refractory state is either only part of the story or that it is an expression of the mechanical-geometrical dynamics of the myosin heads. Any chemical kinetic scheme should also take into consideration the dynamics of the myosin heads and of the active streaming. An alternative purely chemical kinetic model does not have to be sought as a sole substitute to existing models.

Yanagida et al. [5] have reported that when HMM and MgATP are added to F-actin, long filaments are often broken into segments of up to a few micrometers in length, as seen by fluorescence microscopy. This is probably related to the finding [34] that HMM in the presence of MgATP makes the length distribution of F-actin sharper. It is probable that the destabilizing effect discussed above of HMM + MgATP on the subunits at the ends of actin filaments may operate also at other points, leading to easier fragmentation and hence to re-distribution of filament lengths. Thus, the mechanochemically induced increase in the flexibility of actin filaments probably accounts for both the increase in the rate of dissociation and for the stronger inclination to undergo fragmentation. It should, therefore, not be surprising that the addition of tropomyosin, which has been found to cause a decrease in flexibility [1], will both slow down depolymerization by DNase 1 [8] and inhibit the spontaneous fragmentation of F-actin in solution [35].

The enhancement by myosin in the presence of MgATP of the exchange of the nucleotide and of the divalent cation bound to F-actin reported by various authors has been ascribed to "accidental disruption of bonds between actin monomers due to a mechanical stress exerted on actin filaments upon their interaction with myosin filaments" [36]. We may now make this statement more explicit and relate enhanced exchange to the mechanochemically induced increase in the flexibility of actin filaments.

The value of the maximal isometric tension developed by a glycerinated muscle increases upon lowering the ionic strength [37]. If both the capability to generate tension and the increase in flexibility are taken as measures of mechanochemical reactivity we should anticipate that, upon lowering the ionic strength of solutions containing F-actin, HMM and MgATP, the flexibility of the filaments should increase and, along with it, the acceleration induced by DNase 1 of the depolymerization reaction should also be increased. On the other hand, as mentioned above, the extent of binding of myosin heads to F-actin in the presence of MgATP is also larger in low ionic strength media. Thus, according to the view that complexes are also formed between myosin heads and F-actin in the presence of MgATP, the chance for an actin monomer positioned at the end of an actin filament to be in a 'bound' state at any given moment should, intuitively, be higher at low ionic strength and this could lead to diminution of the activity of DNase 1 in depolymerizing F-actin as observed in the presence of HMM and the absence of MgATP [8,9].

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