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Conformational Effects of Cation Binding to Myosin and Their Relation to Phosphorylation†

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ABSTRACT: Binding profiles of calcium or magnesium ions to rabbit skeletal myosin can be determined from the change in the products of chymotryptic digestion with concentration of the ion. Qualitatively similar effects are brought about by sodium ions at much higher concentrations, and the profiles follow expectations for stoichiometric binding of the univalent ion. The affinity for sodium ions depends on the phosphorylation state of the metal-binding ("regulatory") light chain. The degree of saturation at physiological ionic strength is substantially higher for the phosphorylated form than for the unphosphorylated form. This effect can account quantitatively for the apparent difference in affinity for calcium ions between these two states, measured at physiological sodium ion concentration. Thus if phosphorylation of the light chain leads to a structural perturbation linked to the binding of cations, this may be presumed to come about by way of a net change in occupancy of the cation binding sites rather than through displacement of a resident ion by calcium. A similar pattern of behavior is displayed by cardiac myosin. Like calcium or magnesium, sodium ions are found to protect the proteolytically labile sites in the metal-binding light chain itself and at the head–rod junction, and they promote scission in the putative hinge region, with liberation of heavy meromyosin. The digestion pattern is, under the conditions employed here, unaffected by the state of dispersion of the myosin and is thus not dependent on intramolecular contacts in the filamentous form. If the changes in proteolytic sensitivity in the the head region and at the hinge are indeed reflections of the same binding process, it would follow that the conformational effects of attachment of cations to the light chain are transmitted to the hinge, more than 40 nm away. The existence of separate binding processes in the same range of metal ion concentration cannot, however, be excluded. The stability of skeletal myosin filaments with respect to ionic strength is unaffected by phosphorylation, though only if the metal-binding light chains are undamaged. If they are appreciably degraded, the transition between the monomeric and filamentous states is displaced toward higher ionic strength. The dephosphorylated light chain in situ is much more prone than the phosphorylated to adventitious degradation. With chymotrypsin, only the dephosphorylated light chain gives rise (regardless of whether divalent ions are present or absent) to the intermediate product of scission of M_r 17 000 that still contains the metal ion binding site. When the transition between soluble and filamentous myosins is followed by measurements of turbidity, it is consistently found that dephosphorylated myosin gives rise to a greater turbidity, indicative of the formation of structurally altered filaments.

All known myosins contain low molecular weight subunits, or light chains, certain of which bind divalent metal ions. In invertebrate muscle these subunits are implicated in the capture of calcium ions, released on activation of the muscle, and thus in the regulation of contraction (Szent-Györgyi et al., 1973; Lehman & Szent-Györgyi, 1975; Stafford et al., 1979). The isolated light chain possesses a nonspecific binding site, with comparable affinities for calcium and magnesium, whereas the calcium-specific binding process depends on the association of the regulatory light chain with its heavy chain (Bagshaw & Kendrick-Jones, 1979; Chantler & Szent-Györgyi, 1980). In vertebrate skeletal muscle each myosin contains only a nonspecific metal ion binding site, located on the light chain. At calcium concentrations in the range attained during contraction, these sites would in large measure be occupied by calcium; on the other hand, Bagshaw & Reed (1977) have shown that the rate of displacement of the magnesium ions, with which they were presumed to be associated in the absence of calcium, would be too slow to operate within the time scale of a twitch.

A further property of vertebrate skeletal "regulatory" light chains is that, like those of smooth muscle, they are prone to phosphorylation in vivo, the phosphoryl group being introduced and detached by a specific calcium-activated light chain kinase and a phosphatase, respectively (Pires & Perry, 1977; Morgan et al., 1976). Phosphoryl turnover has been shown to accompany the contraction–relaxation cycle (Bárány et al., 1979). By contrast with its effect in smooth muscle [Chacko et al. (1977), but see also Persechini et al. (1981)], phosphorylation of the striated muscle light chain is not required for actin activation of ATPase. It does not change the myosin ATPase (Morgan et al., 1976), though an enhancement of ATPase activation by regulated actin filaments has been reported (Pemrick, 1980). Phosphorylation causes a small but readily measurable diminution in the affinity of the light chain for calcium, though not for magnesium, in both the isolated state (Alexis & Gratzer, 1978) and in situ (Kardami et al., 1980). The functional consequences of these small effects of phosphorylation have yet to be established.

As matters now stand then, the functional role of calcium binding, of phosphorylation, and indeed of the regulatory light chain in general in vertebrate striated muscle is a matter of

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conjecture. The light chain evidently exerts an effect on intrafilament interactions between myosin molecules (Pinset-Härtström & Whalen, 1979). There is also a certain amount of evidence that it in some manner moderates the interaction of the myosin heads with actin (Margossian et al., 1975; Pemrick, 1977; Malhotra et al., 1979; Craig et al., 1980). The indications are that this is not a direct effect but is conformationally mediated. The association of the light chains with calcium or magnesium ions in fact generates a quite large conformational change (Alexis & Gratzer, 1978), which is presumably responsible for the protection of the head–rod junction against proteolysis (Weeds & Pope, 1977; Kardami et al., 1980). Mrâcovčič et al. (1979) have reported that the same type of effect can be engendered by univalent ions at high concentrations. At the same time, at least in the filamentous state, calcium ions promote scission at the "hinge" region of the rod to give heavy meromyosin. This has been generally supposed to be a consequence of the interaction of this part of the rod with the head of an adjoining molecule in the filament.

We have explored the conformational basis of this effect, and we have examined the manner in which phosphorylation affects the relation between the heavy and light chains; the results bear on the possible functions of the divalent cation binding equilibrium and of light chain phosphorylation.

Materials and Methods

Rabbit skeletal myosin was prepared in the phosphorylated and dephosphorylated states by the method of Pires et al. (1974) and in most of the later experiments by the following modified procedure. Crude myosin (Perry, 1955) was prepared by brief extraction of excised back and leg muscles with 3 volumes of 0.3 M potassium chloride, 0.15 M potassium phosphate, 1 mM dithiothreitol, 0.2 mM phenylmethanesulfonyl fluoride, and 0.1 mM sodium azide, pH 7, and precipitation with ice-cold water. It was incubated for 1 h at room temperature in a medium containing 50 mM potassium chloride, 10 mM potassium phosphate, 20 mM sodium glycerophosphate, 12.5 mM magnesium acetate, 5 mM ATP, 2 mM EGTA,[1] 1 mM dithiothreitol, 0.1 mM phenylmethanesulfonyl fluoride, and 0.1 mM sodium azide, pH 7.5, to give dephosphorylated myosin, or in the same solvent containing in addition 0.4 mM calcium chloride (omitting the EGTA) to bring about phosphorylation. At the end of this period the solution was made 10 mM in EDTA. The myosin was then reprecipitated with cold water, and actomyosin was removed by centrifugation at 60000*g* for 1 h in 0.28 M potassium chloride, 10 mM potassium phosphate, 1 mM dithiothreitol, 0.2 mM EDTA, and 0.1 mM phenylmethanesulfonyl fluoride, pH 7, and/or precipitation with ammonium sulfate, retaining the 45–55% cut. Bovine cardiac myosin was prepared from fresh hearts by the method of Tada et al. (1969), followed by ion-exchange chromatography on DEAE-cellulose (Taylor & Weeds, 1976). All preparations were screened for purity by polyacrylamide gel electrophoresis in sodium dodecyl sulfate ($NaDodSO_4$), using a Tris–bicine buffer system (Kendrick-Jones & Jakes, 1976), and for degree of phosphorylation by gel electrophoresis in the presence of 8 M urea (Perrie & Perry, 1970).

Chymotryptic digestions were performed as described before (Kardami et al., 1980) at enzyme:substrate ratios of between 50 and 200 for 5–10 min at 20 °C, the reaction being terminated by addition of phenylmethanesulfonyl fluoride to a concentration of 1 mM. The digests were analyzed, as also previously described, by gel electrophoresis in $NaDodSO_4$ or 8 M urea, followed by staining with Coomassie Brilliant Blue and densitometry. Actin was used as the internal standard, and protein concentrations were kept within the range of linear calibration. Estimations of heavy meromyosin and subfragment-1 were based on calibrations performed with the same proteins. In measuring concentrations of undigested regulatory light chains, the A1 ("essential") light chain was used as the internal reference. Light chain preparations were made by using guanidine hydrochloride to dissociate the myosin, according to Holt & Lowey (1975).

For the measurement of solubility profiles, the myosin was dialyzed against 0.3 M sodium chloride, 10 mM sodium phosphate, 0.2 mM EDTA, and 0.2 mM dithiothreitol, pH 7.2, and clarified by centrifugation at 80000*g* for 2 h. The concentration was determined spectrophotometrically, taking $E^{1\%}_{1cm}$ (280 nm) = 5.5 (Godfrey & Harrington, 1970). Aliquots of this solution were diluted with the same solvent, differing only in its sodium chloride concentration. Equilibrium was reached within 2 h; in general the samples were allowed to stand overnight and then examined by centrifugation, followed by spectroscopic determination of the myosin remaining in the supernatant, or in terms of turbidity in the direction of illumination, using a spectrophotometer (Perkin-Elmer Coleman 575) at 340 nm with cells of 1-cm path length. Myosin concentrations for these experiments were about 2.5 mg/mL.

Results

Dependence of Chymotryptic Digestion on Cations and Phosphorylation State. Limited chymotryptic digestion of myosin causes scission at four sites; two are in the regulatory (DTNB) light chain, one about 17 residues and the other about 43 residues from the N-terminal end, and two are in the heavy chain, one at the junction of the head and the rod (thus giving rise to subfragment-1) and the other at the supposed hinge region of the rod, which generates heavy meromyosin. As established in earlier work (Weeds & Pope, 1977; Kardami et al., 1980; Ritz-Gold et al., 1980), the subfragment-1–subfragment-2 junction and the light chain sites are protected when divalent cations are bound to the light chain and also by high concentrations of sodium ions (Oda et al., 1980). The concentration profile of the protective effect of calcium is displaced toward higher concentrations by phosphorylation of the light chain (Kardami et al., 1980). The digestion of the light chain successively generates fragments of M_r 17 000 and 14 000, the first of which comigrates with the A2 light chain in $NaDodSO_4$ gel electrophoresis, as first recognized by Weeds & Pope (1977). We find in fact that the larger fragment, which retains the metal binding site (Weeds & Pope, 1977), is in our conditions of digestion exclusively a product of the dephosphorylated form (Figure 1), regardless of the salt concentration or the presence of divalent metal ions, and is consistently observed in myosin preparations of high and low levels of phosphorylation; in this respect therefore, since the digestion was performed on soluble monomeric myosin, the light chains of the two heads appear to act independently of one another. Similar results were obtained with cardiac myosin.

Under conditions in which the dephosphorylated light chain almost disappeared, the phosphorylated fraction in a partly phosphorylated myosin preparation was essentially unaffected by chymotrypsin (Figure 1). This specificity did not operate in isolated light chains, where it appears that the phosphory-

[1] Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

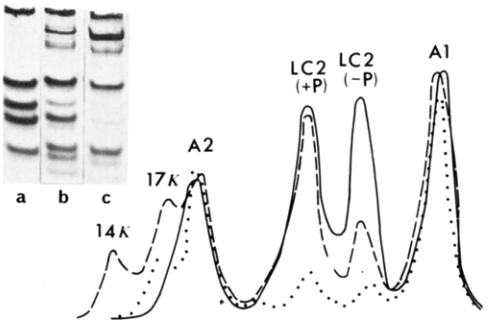

FIGURE 1: Effect of phosphorylation on chymotryptic hydrolysis of metal-binding light chain in situ. Densitometer traces are of stained gels (inset) after electrophoresis in 8 M urea of light chains derived from chymotryptically digested partially phosphorylated myosin (conditions as in text). The full line shows the light chain pattern from the myosin before proteolysis (a). A1 and A2 are the alkali light chains, and the two components of the metal-binding light chain (LC2) refer to the phosphorylated and dephosphorylated forms (+P and −P). The broken line shows the products of proteolysis in the presence of 50 μM calcium ions (b) and the dotted line those in the presence of EDTA (c). (An identical pattern to (b) is obtained in 2 mM magnesium chloride.) Note the disappearance of the dephosphorylated species and almost complete preservation of the phosphorylated species in (b). The products of digestion, which have molecular weights of about 17 000 and 14 000, are indicated. The myosin concentration was 2.5 mg/mL, and digestion was performed for 10 min at an enzyme:substrate ratio of 1:200.

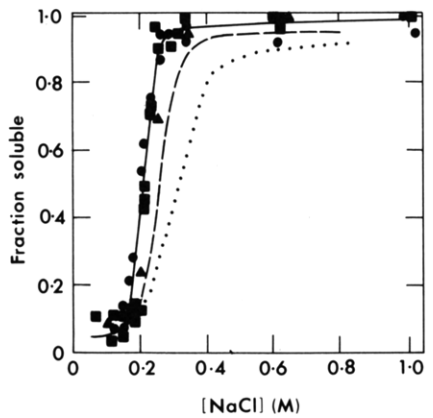

FIGURE 2: Salt concentration–solubility profiles of myosin for fresh preparations of dephosphorylated (●), phosphorylated (■), and partially phosphorylated (▲) proteins. The broken line shows the profile for phosphorylated myosin after storage on ice for 2 weeks or after 2 months at −20 °C in 70% glycerol. The dotted line is for dephosphorylated myosin stored under the same conditions.

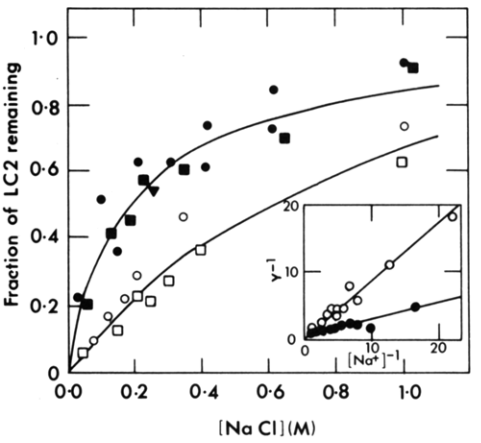

FIGURE 3: Fractional preservation of metal-binding light chains during chymotryptic digestion of myosin as a function of sodium chloride concentration in the absence of divalent cations: phosphorylated light chains in fully (●) and partially phosphorylated (■) myosins; dephosphorylated light chains in fully (○) and partially phosphorylated (□) myosins. Digestion conditions as in Figure 1. (Inset) Linear plots of reciprocal degree of light chain protection against reciprocal sodium ion concentration for phosphorylated (●) and dephosphorylated (○) myosins.

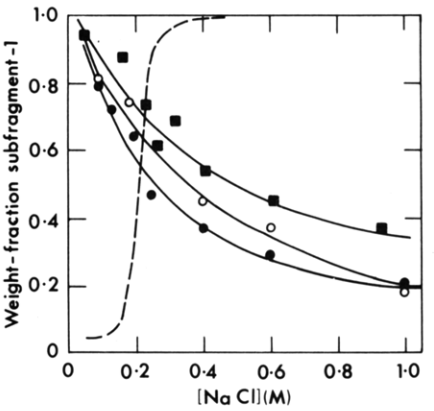

FIGURE 4: Subfragment-1 yield (expressed as a fraction of the total soluble digestion product) from chymotryptic attack on myosin as a function of sodium chloride concentration: phosphorylated (●) and dephosphorylated (○) myosins (2.5 mg/mL) digested at a chymotrypsin:myosin ratio of 1:200 for 10 min. Also shown is the effect of increasing the chymotrypsin:myosin ratio of 1:50 for phosphorylated myosin (■). Under these more severe conditions, the difference between the phosphorylated and dephosphorylated proteins in terms of the digestion yield largely disappears. The broken curve is the solubility profile (Figure 2).

lated state may actually be more labile.

Relation of Protection by Metal Ions to State of Dispersion of Myosin. The solubility profile of myosin as a function of salt concentration is shown in Figure 2. This demonstrates that the ionic strength boundary of the stability of thick filaments is unaffected by the phosphorylation state. It was found essential in establishing this conclusion to use only freshly prepared myosin, for storage (even at −20 °C under ammonium sulfate or in 50% glycerol and in the presence of protease inhibitors) leads to changes in the profile and evidence of appreciable degradation of the metal-binding light chain, as revealed by electrophoresis in 8 M urea. Dephosphorylated myosin is much more sensitive to this form of damage.

Like calcium or magnesium, sodium ions, though of course at much higher concentrations, protect not only the regulatory light chain against degradation but also the head–rod junction, so that the formation of subfragment-1 is suppressed. Concomitantly heavy meromyosin becomes increasingly prominent in the digest. The dependence of light chain protection on the sodium ion concentration as a function of phosphorylation is shown in Figure 3, and that of subfragment-1 yield in Figure 4. As with calcium ions in filamentous myosin, it was found that when heavy meromyosin predominated in the digest the light chains had remained intact, whereas when subfragment-1 was the major component the light chains were largely truncated. The alkali light chains survive in their entirety under all conditions. The curves in Figures 3 and 4 can thus be taken to reflect at least to a large extent the binding of cations to the light chains.

If the sodium ions exert their effect by site binding on the regulatory light chains at the same site as calcium and magnesium, a plot of $\bar{Y}/[Na^+]$ vs. $[Na^+]$ [where $\bar{Y}$ is a quantity, such as the fraction of light chain undigested, that can be taken (Kardami et al., 1980) to be proportional to the fractional saturation of binding sites] should be linear and may be used to give the binding constant. Linear plots are indeed obtained (Figure 4, inset) and yield binding constants of 6 and 1 M^{-1}, respectively, for the phosphorylated and dephosphorylated

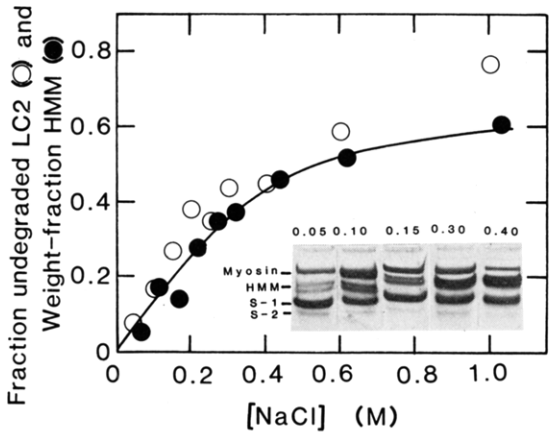

FIGURE 5: Yield of heavy meromyosin (expressed as a fraction of the total soluble digestion product) from chymotryptic digestion of myosin as a function of sodium chloride concentration (●). The myosin in this experiment was partly phosphorylated and was digested at a concentration of 2.5 mg/mL by chymotrypsin at 1:50 for 10 min. Also shown for comparison is the fraction of metal-binding light chains remaining intact (○). (Inset) Electrophoretic gels (run in the presence of $NaDodSO_4$) of myosin digestion products at different sodium chloride concentrations. Note the presence of trace amounts only of subfragment-2 throughout.

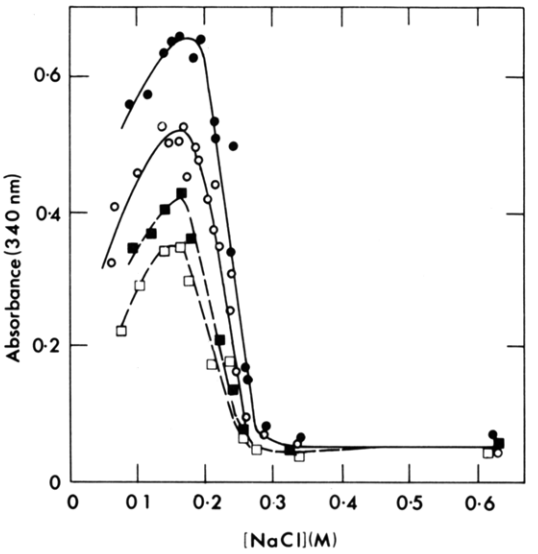

FIGURE 6: Turbidity of myosin solutions (expressed as scattering intensity in the direction of illumination at 340 nm) as a function of sodium chloride concentration: dephosphorylated (●) and phosphorylated (○) myosins in the absence of ATP; dephosphorylated (■) and phosphorylated (□) myosins in the presence of 2 mM Mg-ATP. Myosin concentration 2.5 mg/mL throughout.

states. Thus the relative affinities of these two forms are in the opposite sense to those measured for calcium [apparent binding constants at physiological ionic strength of 4×10^6 and 8×10^6 M^{-1}, respectively (Kardami et al., 1980)]. The calcium binding constants were obtained from plots of relative saturation of sites against pCa (Kardami et al., 1980). At the midpoint of this profile with competition at the same site

$$K_{Na}[Na^+] - K_{Ca}[Ca^{2+}] = 1$$

where K_{Na} and K_{Ca} are true binding constants. With the above values for K_{Na}, the calcium binding constants for the phosphorylated and dephosphorylated states become 7×10^6 and 9×10^6 M^{-1}, respectively. They may thus well be the same or at all events are scarcely to be distinguished.

It should be remarked that the profiles under these conditions of digestion are smooth, and there is no evidence that the yield of fragments is dependent on the solubility state of the protein. A discontinuity in the region of ionic strength in which the myosin filaments dissociate can be detected only at very low enzyme concentrations, at which there is little digestion; here some inhibiting effect of dissociation into filaments evidently shows itself. With regard to the data of Figures 3 and 4, however, it seems clear that protection results essentially entirely from ion binding. The same can be inferred from the profile relating the concentration of heavy meromyosin in the digest with sodium ion concentration (Figure 5). In this experiment a higher enzyme concentration was used to eliminate the difference in sensitivity between the phosphorylated and dephosphorylated forms. The electrophoretic gels in Figure 5 (inset) show that over the whole range of salt concentration only trace amounts of subfragment-2 were formed. Moreover the total yield of soluble digest estimated from such gels in all experiments was invariant throughout the range within the experimental accuracy (better than 5%).

Experiments with cardiac myosin showed a similar change in digestion pattern with salt concentration, and its light chains will thus evidently bind univalent ions in the same way as those in skeletal myosin.

Effects of Phosphorylation on Filament Formation. Phosphorylation of the light chain has, as already remarked, no significant effect on the equilibrium between myosin and its filaments. However, when aggregation of myosin was measured in terms of light scattering in the spectrophotometer (Figure 6), it was consistently found that the peak of turbidity, which occurred at a sodium chloride concentration of 0.16 M, was higher for the dephosphorylated form. Below this salt concentration the measured turbidity dropped, presumably by reason of formation of large aggregates. Whether dephosphorylated myosin forms filaments of greater average length than phosphorylated or produces filaments differing appreciably in their flexibility or their tendency to associate with each other is not yet clear. Preliminary inspection in the electron microscope revealed no gross differences. The solubility profiles were unaffected by calcium or magnesium ions at millimolar concentrations, but the turbidity was not unexpectedly reduced by 2 mM Mg-ATP and the profile shifted to slightly lower salt concentrations.

Discussion

Our results indicate, in agreement with Oda et al. (1980), that in regulatory light chains of skeletal myosin, both in the isolated state and in situ, the conformational effects of divalent cations can be reproduced with sodium ions at high concentrations. Our data (Figure 3) suggest that the sodium ion may interact according to a true binding equilibrium, in competition with divalent ions at the single strong site. This evidently applies also to cardiac myosin. There is a considerable difference in affinity for sodium ions between the phosphorylated and dephosphorylated light chains, which is opposite in sense to their apparent affinities (at physiological ionic strength) for calcium and magnesium ions (Alexis & Gratzer, 1978; Kardami et al., 1980). These shifts in measured affinity for divalent cations and the difference in the extent of proteolytic scission in the phosphorylated and dephosphorylated states, which is large in the absence of divalent cations and has been interpreted in terms of a conformational differences (Ritz-Gold et al., 1980), can now be substantially explained in terms of different degrees of saturation of the binding sites in the two cases by sodium.

The profiles of Figures 3–5 show that at least under these experimental conditions there is no significant effect of the state of dispersion of the myosin on the conformational

characteristics that govern the sensitivity of the proteolytically labile sites. These are evidently regulated by an interaction with sodium ions, qualitatively resembling that with calcium and magnesium. Both univalent and divalent cations evidently exercise a direct effect on the light chains to which they bind and on the head–rod junction, as well as a parallel inverse effect on the proteolytic susceptibility of the hinge region. The independent effect at the hinge of calcium ions was demonstrated by Weeds & Pope (1977) for filamentous myosin. That sodium ions indeed operate in an analogous manner (regardless of whether the protein is filamentous or monomeric) is suggested by the observation that there is no significant increase in the amount of subfragment-2 appearing in the digest or in the total fragment yield with diminishing salt concentration (Figure 5), for if the subfragment-1 generated in these conditions were due to breakdown of heavy meromyosin, then an equivalent amount of subfragment-2 would be liberated, and the overall yield would also rise by reason of the increased lability of the head–rod junction. Thus one interpretation of our data is that the binding of a cation to a site on the regulatory light chain, which is evidently located at the junction of the head and the rod [see, e.g., Kuwayama & Yagi (1980); Craig et al., 1980], causes a conformational change, which is transmitted to the hinge region, about 43 nm down the rod (Elliott & Offer, 1978). We cannot exclude, however, that the parallelism between the different scission processes is fortuitous [though this is scarcely possible in the case of calcium ions, for which the light chains are the only strong binding sites (Bagshaw, 1977)] and that there are specific sodium binding sites with the same affinity as those on the light chains in the hinge region. Some evidence of multiple site binding of sodium ions to myosin exists (Lewis & Saroff, 1957).

The function of the regulatory light chains in thin-filament-regulated muscle is still far from clear. Although the assembly of the thick filament is predominantly a function of the myosin rod, in smooth muscle (Suzuki et al., 1978) and especially nonmuscle myosin (Scholey et al., 1980), a change in the head region, or rather at the head–rod junction, resulting from phosphorylation of the regulatory light chain, can have profound effects on filament formation. Our results show no such influence on the stability of the filaments formed by skeletal myosin, at least with respect to salt concentration, but the dependence of turbidity on phosphorylation (Figure 6) must be taken to reflect a structural disturbance. That the light chain affects thick-filament structure is evident from the work of Pinset-Härström & Whalen (1979), and we find, in agreement with their observations, that reproducibility of the results is critically dependent on integrity of the light chains and thus on the use of fresh myosin preparations, especially in the dephosphorylated form. Pinset-Härström & Truffy (1979) have furthermore found that calcium and magnesium have a regulatory effect on thick-filament structure and that the size of synthetic filaments depends on the identity of the divalent cations present.

The relation between the regulatory light chain and the state of the myosin filament is presumably related to the orientation of the heads with respect to the rod or to their freedom to rotate or swivel about the subfragment-1–subfragment-2 junction. This may be supposed also to underly the influence of the light chain on the interaction with actin, as reflected in the binding affinity in solution (Margossian et al., 1975), the geometry of the interaction with actin filaments (Craig et al., 1980), and the actin activation of the ATPase activity (Pemrick, 1977; Malhotra et al., 1979), all of which are changed when light chains are removed. A possible mechanism for at least some of these phenomena may involve an increased tendency for association of heads when one of the two regulatory light chains is lost (Bagshaw, 1980). It seems most likely therefore that the sizable conformational changes, to which the light chains are subject on binding of ions (Werber et al., 1972; Werber & Oplatka, 1974; Alexis & Gratzer, 1978; Mrâcovčič et al., 1979) and which in turn are moderated by phosphorylation, also serve to modify the interaction with the actin filaments. This supposition is consistent with the observation by Kopp & Bárány (1979) that myosin phosphorylation (in heart muscle) manifests itself only in a change in active tension.

Our results thus suggest a mode of action of the metal binding sites of skeletal myosin, more plausible perhaps than partial displacement of magnesium by calcium following activation [which is known to be slow (Bagshaw & Reed, 1977)]. This takes the form of a change, consequent on phosphorylation or dephosphorylation, in the occupancy of the metal ion binding sites by sodium or magnesium with an ensuing conformational readjustment, causing in turn a steric modification of the interaction between the myosin heads and the thin filaments. This may be desirable for the maintenance of tension under conditions of continuing stimulation. Under these circumstances large changes in the degree of phosphorylation have been shown to occur (Bárány et al., 1979). In energetic terms the difference in cation affinity, and probably also affinity of the heads for actin, is small. However, if cooperativity of interaction of myosin heads with actin is present (Trybus & Taylor, 1980), such a small change in affinity can effect a large shift in the overall extent of interaction. [For a remarkable example of such a phenomenon in a different binding system involving actin, see Walsh & Wegner (1980).]

The regulatory light chain is thought to be an elongated molecule (Stafford & Szent-Györgyi, 1978; Alexis & Gratzer, 1978), the cation binding site being at the same (N-terminal) end as the phosphorylation and proteolysis sites (Collins, 1976). The conformational change induced by the bound cation evidently affects the C-terminal part, to judge by the spectroscopic perturbation of the aromatic residues clustered in this region (Werber et al., 1972; Werber & Oplatka, 1974; Alexis & Gratzer, 1978). This end is apparently located in the myosin head (Kendrick-Jones & Jakes, 1976) and is probably spatially remote from the N terminus. The light chain may thus perhaps be seen as a transducing link between parts of the myosin and hence as a determinant of the preferred geometry in a region of considerable structural freedom.

Acknowledgments

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