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Effects of Actin and Calcium Ion on Chymotryptic Digestion of Skeletal Myosin and Their Implications to the Function of Light Chains†

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ABSTRACT: Experiments have been carried out to assess the involvement of the myosin light chains [obtained by treatment of myosin with 5,5′-dithiobis(2-nitrobenzoic acid) (Nbs_2)] in the control of cross-bridge movement and actomyosin interactions. Chymotryptic digestions of myosin, actomyosin, and myofibrils do not detect any Ca^{2+}-induced change in the subfragment 2 region of myosin. Actin, like Ca^{2+}, protects the in situ Nbs_2 light chains from proteolysis and causes a partial switch in the digestion product of myosin from subfragment 1 to heavy meromyosin. This effect is independent of the state of aggregation of myosin, and it persists in acto heavy meromyosin and in actomyosin in 0.6 M NaCl. Digestions and sedimentation studies indicate that there is no direct acto light chain interaction. Proteolysis of myosin shows a gradual transition from production of heavy meromyosin to subfragment 1 with lowering of the salt level. In the presence of Ca^{2+} heavy meromyosin is generated both in digestions of polymeric and of monomeric myosin. These results are explained in terms of localized changes within the Nbs_2 light chains and subfragment 1. Subunit interactions in the myosin head lead to a Ca^{2+}-induced reduction in the affinity of heavy meromyosin for actin in the presence of MgATP. The resulting Ca^{2+} inhibition of the actin-activated ATPase of myosin can be detected at high salt concentrations (75 mM KCl).

The movement of myosin cross bridges is a central feature in all of the theories of muscular contraction (Huxley, 1969; Huxley & Simmons, 1971; Eisenberg & Hill, 1978; Harrington, 1979a). The nature and the mechanical consequences of this movement have not been yet fully elucidated. In the Huxley model (Huxley, 1969), the action of the cross bridges is linked to a "swinging away" of the subfragment-2 (S-2)[1] region of myosin from the backbone of the thick filament. A transient release of the S-2 region is similarly postulated in the helix–coil model of force generation in muscle (Harrington, 1971, 1979a). Several studies support the view that some conformational changes occur in the thick filament when Ca^{2+} binds to the associated Nbs_2 chains. These Ca^{2+}-induced changes were detected by hydrodynamic experiments with thick filaments (Morimoto & Harrington, 1974) and X-ray diffraction studies with striated muscle (Haselgrove, 1975; Huxley, 1979). The binding of Ca^{2+} also appears to significantly weaken actomyosin interactions (Margossian et al., 1975). The most striking Ca^{2+} effect is revealed in the chymotryptic digestions of polymeric myosin. Subfragment 1 is produced in the absence of divalent cations, and heavy meromyosin, in their presence (Weeds & Taylor, 1975; Weeds & Pope, 1977). These and other observations form a basis for a speculation that the Nbs_2 light chains could be involved in the structural regulation of the myosin cross-bridge movement (Haselgrove, 1975; Harrington, 1979b). However, more direct tests of the mobility and disposition of myosin heads with respect to the thick filament backbone do not confirm a specific Ca^{2+}-induced action (Mendelson & Cheung, 1976; Sutoh & Harrington, 1977). Also, more recent work of Srivastava et al. (1980) does not detect any involvement of the Nbs_2 light chains in the generation of tension by actomyosin threads.

The best documented function of myosin light chains is the modulation of the actin-activated MgATPase in invertebrate muscles (Kendrick-Jones et al., 1976). No equivalent Ca^{2+} sensitivity has been found in vertebrate skeletal muscle. However, activity measurements in solvents approaching physiological ionic strengths may produce a different picture. Thus, removal of the Ca^{2+} binding subunit from cardiac myosin results in an increase in actin-activated ATPase at elevated salt concentrations (Malhotra et al., 1979). In rabbit myofibrils, free of troponin and tropomyosin, Ca^{2+} activation of the MgATPase activity has been detected in solutions containing more than 80 mM KCl (Lehman, 1978). This suggested that perhaps in the typical assays, at low ionic concentrations, the affinity of myosin for actin overrides the Ca^{2+} dependence (Lehman, 1978).

In this work we have attempted to reconcile some of the seemingly inconsistent observations related to the function of the Nbs_2 light chains by investigating the interrelationship between actin and the myosin heavy and light chains. Chymotryptic digestions of myosin, acto heavy meromyosin, actomyosin, and myofibrils revealed a significant actin-induced

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[1] Abbreviations used: ATPase, adenosine triphosphatase; Nbs_2 (DTNB in figures) light chain, 19000 molecular weight subunit of myosin dissociated by treatment with 5,5′-dithiobis(2-nitrobenzoic acid); HMM, heavy meromyosin; S-1, subfragment 1; S-2, subfragment 2; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; DTE, dithioerythritol; ATP, adenosine 5′-triphosphate; EDTA, ethylenediaminetetraacetic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; Bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol.

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protection of the light chains from proteolysis. We found no direct interaction of actin with the isolated Nbs_2 chains. The observed digestion patterns of polymeric myosin are probably related to local changes in the myosin head and the Nbs_2 chains and not to any significant transition in the S-2 region of the myosin molecule. Assays of actin-activated MgATPase, carried out in 75 mM KCl, have detected a slight inhibition of activity upon the addition of Ca^{2+} cations. This effect probably reflects a Ca^{2+}-induced change in the affinity of myosin for actin.

Materials and Methods

α-Chymotrypsin, phenylmethanesulfonyl fluoride, EGTA, DTE, and ATP were obtained from Sigma Chemical Co. (St. Louis, MO). Cibacron blue F3GA covalently attached to cross-linked agarose (Affi-Gel Blue) was purchased from Bio-Rad Laboratories. All other reagents were of analytical grade.

Preparation of Proteins. Myosin was prepared from rabbit skeletal muscle according to the procedure of Godfrey & Harrington (1970). It was stored in 0.5 M KCl, 0.01 M EDTA, 0.05 M Tris-HCl, pH 7.9, and 50% glycerol at −20 °C. Before use, the myosin was exhaustively dialyzed against 0.6 M NaCl, 10 mM sodium phosphate (pH 7.0), and 0.1 mM NaN_3 and clarified by centrifugation at 100000*g* for 2 h.

Heavy meromyosin (HMM) was prepared by digesting a 15 mg/mL myosin solution in 0.6 M NaCl, 1 mM $CaCl_2$, and 10 mM sodium phosphate (pH 7.0) for 10 min. The digestion was conducted at room temperature, with α-chymotrypsin at a final concentration of 0.05 mg/mL (Weeds & Taylor, 1975). Digestion was terminated by adding phenylmethanesulfonyl fluoride to a final concentration of 1.5 mM. HMM was isolated and purified from the digestion products as described by Weeds & Pope (1977). Subfragment 1 (S-1) was obtained by chymotryptic digestion of myosin in 0.12 M NaCl, 1 mM EDTA, and 10 mM sodium phosphate (pH 7.0) for 10 min. Digestion and purification followed previously described procedures (Weeds & Taylor, 1975; Reisler, 1980).

Total light chains of myosin were prepared by their dissociation from the heavy chains in 5 M guanidine hydrochloride, followed by an ethanol precipitation of the heavy chains (Holt & Lowey, 1975). The Nbs_2 chains were purified and isolated as a homogeneous fraction by chromatography of the light chain preparation on an Affi-Gel Blue (Cibacron blue) column (Mrakovčič et al., 1979; Toste & Cooke, 1979). Myofibrils were made following the method of Kominz (1970).

Actin free of troponin and tropomyosin was prepared by using the method of Spudich & Watt (1971). The extraction of actin from the acetone powder was conducted for 2 h at pH 7.5, and at 4 °C. At least two cycles of polymerization–depolymerization were employed. Prior to its combination with myosin or HMM, actin was polymerized in the appropriate digestion buffer, in the presence of $MgCl_2$ (1.0 mM).

Chymotryptic Digestions. These digestions were carried out at room temperature, in 10 mM sodium phosphate buffer (pH 7.0) and 0.1 mM NaN_3, in the presence of 0.6 M NaCl (high salt), 0.36 M NaCl, or 0.12 M NaCl (low salt). Myosin was digested at 6 mg/mL in the presence of 1.0 mM $CaCl_2$ or EDTA by using α-chymotrypsin at 0.05 mg/mL. Reaction times varied from 2 to 10 min. The reactions were terminated by adding phenylmethanesulfonyl fluoride (final concentration, 1.5 mM). Actomyosin solutions, which were digested under identical conditions, contained 6 mg/mL myosin and 2 mg/mL F-actin. HMM was digested at a concentration of 3 mg/mL, and the acto-HMM used in these studies consisted of 3 mg/mL HMM and 1 mg/mL actin. Myofibrils were digested at a total protein concentration of 7 mg/mL, determined by a biuret assay.

The Nbs_2 light chains were digested at 0.5 mg/mL, for 30 s, by employing 5 μg/mL α-chymotrypsin. The reaction was carried out at room temperature, in 0.12 M NaCl, 10 mM sodium phosphate (pH 7.0), and 1 mM EDTA. When added to the digestion system, actin was present at a threefold molar excess over the Nbs_2 light chains.

Whenever required, the digested proteins were dialyzed against 40 mM NaCl and 5 mM sodium phosphate (pH 6.5) and clarified by centrifugation at 40000*g* for 1 h. This procedure removed the undigested and insoluble components. The soluble products of the supernatant were examined on sodium dodecyl sulfate gel electrophoresis.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Gel electrophoresis was carried out according to the procedure of Laemmli (1970) by using a two-phase resolving gel (10% and 15% acrylamide). The protein distribution among the different bands was quantitated as described before (Mrakovčič et al., 1979).

Binding Studies. Binding of the Nbs_2 light chains to F-actin was tested in a Model E analytical ultracentrifuge equipped with a photoelectric scanning system. In these experiments we followed the procedures of Margossian & Lowey (1973, 1978) and Reisler (1980). Actin (2 mg/mL) was combined with the Nbs_2 light chains (1.0 mg/mL) in 0.1 M KCl, 3 mM $MgCl_2$, and 25 mM Bis-Tris buffer (pH 7.5) and sedimented at 40000 rpm at 21 °C. No cosedimentation of the Nbs_2 light chain with actin could be detected.

Measurements of ATPase Activity. The actin-activated ATPase of HMM and S-1 was measured at 23 °C in 0.01 M Tris–histidine (pH 7.5), 3 mM $MgCl_2$, 2 mM ATP, and 12 or 75 mM KCl. Either $CaCl_2$ (1 mM) or EGTA (1 mM) was included in the incubation mixtures. HMM or S-1 was present at concentrations of 0.05–0.1 mg/mL; actin concentrations ranged from 0.3 to 3.5 mg/mL. The activity measurements were carried out as previously described (Reisler, 1980), and the results were analyzed by Lineweaver–Burk plots with F-actin regarded as the substrate. Enzyme activities are reported as turnover rates in molar units [μmol of P_i (μmol of active sites)$^{-1}$ s^{-1})].

Results

Effect of Actin on the Nbs_2 Light Chains. Chymotryptic proteolysis of monomeric myosin (high salt) results in the production of heavy meromyosin (HMM). Digestion of polymeric myosin (low salt) can yield two types of products. Subfragment 1 (S-1) is produced in the absence of divalent cations; HMM is generated in their presence (Weeds & Pope, 1977). The amount of HMM obtained from polymeric myosin is rather small compared to its yield obtained in digestions of monomeric myosin (Weeds & Pope, 1977). The reduced HMM production in the polymeric myosin could be due to the protection afforded to the HMM site by adjacent molecules of the superstructure. It might be expected that the release of cross bridges from the filament's backbone or the melting of the S-2 region of the molecule would facilitate the proteolysis at the HMM site. (The HMM site is the proteolytically sensitive region in the S-2 fragment.)

Limited chymotryptic digestions of actomyosin (in low salt) yield the same products, S-1 and HMM, as the comparable digestions of myosin alone. Surprisingly, digestions of actomyosin, carried out in the absence of divalent cations (in low salt), produce a substantial amount of HMM, compared to an almost exclusive generation of S-1 from myosin (Table I). At short actomyosin digestion times the ratio of HMM to S-1

Table I: Products of Chymotryptic Digestion of Myosin and Actomyosin[a]

system	ligand (1 mM)	HMM (%)	S-1 (%)	digestion[b] of myosin (%)	protection[c] of Nbs_2 light chains
myosin	EDTA	6	94	40	–
	Ca^{2+}	95	5	16	+
actomyosin	EDTA	59	41	25	++
	Ca^{2+}	96	4	14	+++

[a] The digestions were carried out for 10 min, at room temperature, in 0.12 M NaCl and 10 mM sodium phosphate (pH 7.0) in the presence of either 1 mM EDTA or 1 mM $CaCl_2$. The final protein concentrations were myosin 6 mg/mL, actin 2 mg/mL, and α-chymotrypsin 0.05 mg/mL. The relative amounts of products were quantitated from tracings of sodium dodecyl sulfate–polyacrylamide gels of the soluble products. [b] The percentage digestion refers to the total yield of HMM and S-1 derived from gel tracings. [c] The degree of protection of the Nbs_2 light chain from proteolysis was estimated by visual inspection of gels.

approaches 2.5:1. Along with the generation of HMM, a strong protection of the Nbs_2 light chains is observed (Table I). The protective effect of actin appears to be stronger than that due to binding of Ca^{2+} to myosin alone (Table I). This difference is further accentuated by the fact that virtually no S-1 is produced in Ca^{2+}–myosin digestions, whereas a noticeable amount of such product can be detected in the EDTA–actomyosin digestion. Apparently S-1 can be generated from the actomyosin system without prior degradation of the Nbs_2 light chains. The presence of Ca^{2+} during proteolysis of actomyosin leads to an additional protection of the Nbs_2 chains and an exclusive generation of HMM. The amount of HMM so produced is about the same as in Ca^{2+}–myosin digestions (Table I). Thus, the binding of actin to myosin does not appear to increase the proteolytic susceptibility of the HMM sites. In the absence of divalent cations, the lower amount of actomyosin cleavage products (Table I) is probably due to an actin-induced protection of the S-1 site. Similar effects of actin on the digestion of myosin were also observed in native actomyosin and myofibrils.

Digestions of actomyosin in high salt (0.6 M NaCl, soluble myosin) and acto-HMM in low salt (0.12 M NaCl) provide further evidence for localized perturbations around the Nbs_2 light chains. Extensive digestions of monomeric myosin in the absence of divalent cations yield, along with HMM, the eventual degradation of the Nbs_2 light chains and the appearance of S-1 as a minor product [Figure 1a,b; see also Weeds & Pope (1977)]. Similar digestions of actomyosin reveal significantly reduced cleavage at the S-1 site (Figure 1). A parallel protection of the Nbs_2 light chains is noted in the acto-HMM system. Without divalent cations (in 0.12 M NaCl) the light chains of isolated HMM are rapidly degraded prior to its conversion into S-1 [Figure 1c–e; see also Weeds & Pope (1977)]. In the presence of actin alone the light chains are protected, again more effectively than by bound Ca^{2+} (Figure 1c–e). The observed protection of the Nbs_2 light chains is at the level of monomeric myosin both in the case of acto-HMM and in that of actomyosin in high salt. We anticipate that the same mechanism is responsible for the resultant digestion patterns of actomyosin and myofibrils in low salt.

It appears that the binding of actin to the myosin heavy chains protects the Nbs_2 light chains indirectly, via subunit interactions. We have not detected any direct interaction between actin and the isolated Nbs_2 light chains either in chymotryptic digestions or in sedimentation velocity experiments.

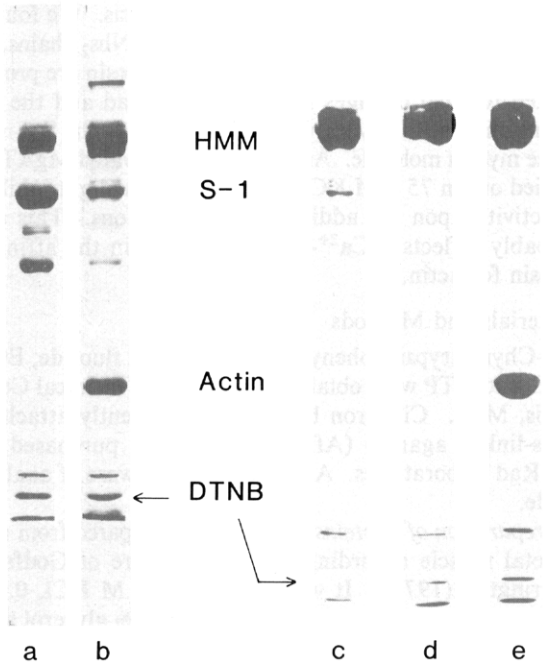

FIGURE 1: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the chymotryptic fragments of myosin. Fragments were run on composite 10 and 15% polyacrylamide gels. (a) Myosin (6 mg/mL) was digested for 5 min, in 0.6 M NaCl, 10 mM phosphate, and 1 mM EDTA, at room temperature, with 0.05 mg/mL chymotrypsin; (b) myosin and the digestion conditions were as described in (a), and actin (2 mg/mL) was combined with myosin prior to proteolysis; (c) HMM (3 mg/mL) was digested for 10 min, in 0.12 M NaCl, 10 mM phosphate buffer (pH 7.0), and 1 mM EDTA, at room temperature, with 0.05 mg/mL chymotrypsin; (d) conditions were as described in (c) except for substitution of 1 mM $CaCl_2$ for EDTA; (e) conditions were as described in (c) but after combining HMM (3 mg/mL) with actin (1 mg/mL).

Ca^{2+} "Sensitivity" of Actomyosin ATPase. Since actin indirectly affects the stability and possibly the conformation of the Nbs_2 light chains, conversely it may be expected that the conformational state of the light chains could influence actomyosin interactions. Indeed, Margossian et al. (1975) have found that the binding affinity of HMM for actin is significantly reduced in the presence of Ca^{2+} (in 0.1 M KCl and 10 mM imidazole, pH 7.0, solvent). Activity assays of actomyosin have not detected a similar Ca^{2+}-induced reduction in the binding of these proteins in the presence of ATP. Such tests are normally conducted under nonphysiological low-salt conditions, where the strong affinity of myosin for actin may override any modulating effect of Ca^{2+} [e.g., Lehman (1978)]. MgATPase activities of acto-HMM measured in 75 mM KCl show a small but consistent lowering of activity in the presence of Ca^{2+}. Similar observations have been made by Bremel & Weber (1975) and Pemrick (1976). Lineweaver–Burk plots of our data yield the same turnover rate per site for the acto-HMM complex (V_{max} = 7.1 s^{-1}) in the absence or in the presence of Ca^{2+} but extrapolate to different apparent dissociation constants of actin from HMM (K_{app}) for the two sets of measurements. Higher K_{app} values are obtained in the presence of Ca^{2+} ($K_{app}(Ca^{2+})$ = 175 μmol; K_{app}(EGTA) = 125 μmol), indicating that Ca^{2+} reduces actomyosin interactions in the presence of MgATP. Activity assays of acto subfragment 1 (containing no Nbs_2 light chains) in 75 mM KCl and measurements of acto-HMM at 12 mM KCl detect no Ca^{2+} sensitivity.

Salt as a Probe in Proteolysis of Myosin. The results of our actomyosin digestions point to a local, probably indirect, actin protection of the Nbs_2 light chains and S-1 cleavage site.

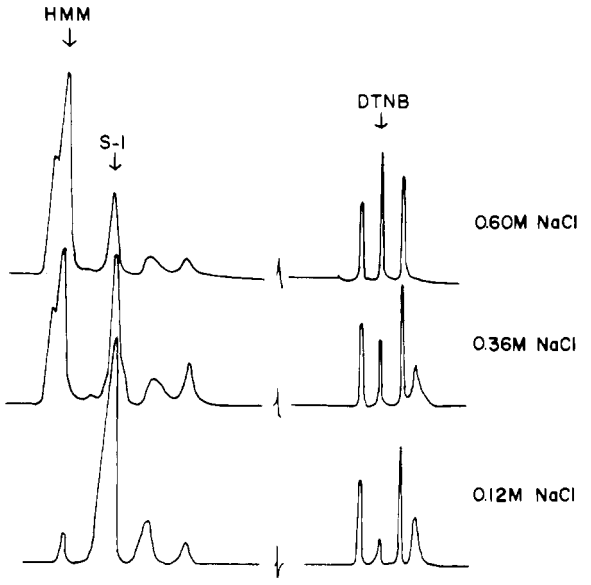

FIGURE 2: Densitometric tracings of sodium dodecyl sulfate–polyacrylamide gels of water-soluble products of myosin digestions. The break in the tracings corresponds to the 10% polyacrylamide–15% polyacrylamide interphase. Myosin was digested with 0.05 mg/mL chymotrypsin, for 10 min, at room temperature, in 10 mM phosphate buffer (pH 7.0) and 1 mM EDTA, at the salt concentrations specified in the figure.

Is the Ca^{2+}-induced switch from S-1 to HMM production during proteolysis of polymeric myosin due to a similar local change in the Nbs_2 light chains or to a structural transition in the S-2 region of the molecule? The effect of salt on the digestion of myosin sheds some light on this question. In a previous work (Mrakovčič et al., 1979), we have examined the effect of salt on the chymotryptic digestion of isolated Nbs_2 light chains. We have found that high concentrations of monovalent cations protect the Nbs_2 light chains from proteolysis and induce conformational changes similar to those caused by the binding of Ca^{2+}. Digestions of myosin at different salt concentrations indicate that the protection of the light chains by salt occurs also in situ (Figure 2). As shown in Figure 2, lowering the salt concentration from 0.6 to 0.36 M NaCl, in the absence of divalent cations, results in the increased production of S-1. This change is noteworthy, since no large polymeric structures are present under 0.36 M NaCl conditions, and, thus, the reduction in susceptibility of the HMM site due to superstructure can be hardly expected. Equally instructive are the digestions of myosin in 5 mM pyrophosphate buffer (pH 8.0) which contains no monovalent salt. In this solvent, myosin exists in a dissociated form (Harrington & Himmelfarb, 1972). In our own preparations, myosin (at 5 mg/mL) sedimented in the pyrophosphate buffer at the rate of 5.5 S. Limited chymotryptic digestions of myosin in the pyrophosphate in the absence of divalent cations yield primarily S-1 (80–90%) and a minor HMM fraction (10–20%). Formation of HMM, in this case, indicates simultaneous digestion at both sites, with the unprotected S-1 site having a higher reactivity. In the presence of Ca^{2+}, HMM is the exclusive product of such digestion. Thus, in the absence of an organized myosin superstructure, the binding of Ca^{2+} to the Nbs_2 light chains leads to a substantial protection of the S-1 cleavage site.

Discussion

Binding of Ca^{2+} and Mg^{2+} to the isolated Nbs_2 light chains results in relatively large changes in this protein. The overall shape of the Nbs_2 light chains becomes more symmetric, the α-helical content increases, and certain spectral properties change as well (Alexis & Gratzer, 1978; Mrakovčič et al., 1979). Paramagnetic resonance studies of bound Mn^{2+} ions reveal that identical spectra are obtained in the isolated and in situ Nbs_2 light chains (Bagshaw & Reed, 1977). Also, small changes can be detected in the hydrodynamic properties of myosin filaments upon the binding of Ca^{2+} (Morimoto & Harrington, 1974). A search for the functional significance of these changes is obviously warranted, although previous results have not proved conclusive. The possibility that the Nbs_2 light chains may be involved in structural regulation of cross-bridge disposition in vertebrate muscle has been considered in several laboratories [e.g., Werber et al. (1972), Haselgrove (1975), Morimoto & Harrington (1974), and Mendelson & Cheung (1976)]. The Ca^{2+} effect on the chymotryptic digestion of polymeric myosin could be consistent with such an idea, if indeed binding of Ca^{2+} leads to a structural transition in the S-2 region of the molecule (Weeds & Pope, 1977). However, recent cross-linking experiments could not detect any Ca^{2+}-induced movement of cross bridges (Sutoh & Harrington, 1977).

Our results of chymotryptic digestions of actomyosin and proteolysis of myosin under different salt conditions suggest that the Ca^{2+} effect is localized in the Nbs_2 light chains and the S-1 part of myosin. Such local effects are demonstrated in digestions of acto-HMM in which the thick filament superstructure is not present. Nevertheless, F-actin efficiently protects the myosin-associated Nbs_2 light chains from cleavage. A direct consequence of such protection is the masking of S-1 digestion site from proteolysis. The net result of this chain of events in actomyosin and myofibrils (in low salt) is the substantial production of HMM during chymotryptic digestions in the absence of divalent cations. The low yield of HMM from actomyosin (low salt and in the presence of Ca^{2+}) argues against a hypothetical Ca^{2+}-induced release of myosin cross bridges from the filament backbone. Similar levels of HMM could be recovered from the proteolysis of myosin or actomyosin, suggesting that the environment of the HMM site in both systems is equivalent.

The effect of actin on the digestion of myosin can be explained in terms of localized changes within the Nbs_2 light chains. A similar interpretation could be applied to the Ca^{2+}-induced transition in the myosin digestion reaction. This argument is supported by examining the salt effect on the digestion of myosin. A clear transition from production of HMM to S-1 can be noticed upon lowering of the salt level (Figure 2). The transition in digestion products is paralleled by the increase proteolysis of the isolated Nbs_2 light chains (Mrakovčič et al., 1979) and is not uniquely related to the state of myosin aggregation. It appears that these products are determined by a combination of several factors: the reaction rates at the HMM and S-1 sites, Ca^{2+} and salt protection of the Nbs_2 light chains and S-1 site, and the organization of myosin in the polymerized state. The Ca^{2+} switch in the digestion products (from S-1 to HMM) can be observed also during proteolysis of myosin in the pyrophosphate buffer, in which no polymers can be detected by analytical ultracentrifugation. We suggest the following explanation for the digestion of myosin filaments: In the filamentous form the HMM sites are sterically hindered from proteolytic attack by adjacent myosin molecules. In the presence of Ca^{2+}, the stabilized Nbs_2 light chains protect the S-1 site from a rapid attack leading to a preferential proteolysis at the HMM junction. Thus, neither Ca^{2+}'s nor actin's effect on digestion should be construed as following the departure of cross bridges

from the filament backbone or transitions in the S-2 region of the molecule. This view of the digestion results is in agreement with the cross-linking experiments which did not detect a Ca^{2+}-induced cross-bridge movement (Sutoh & Harrington, 1977).

The digestions of actomyosin reveal interdependence between actin and the Nbs_2 light chains. Our results are consistent with previously observed Ca^{2+} inhibition of actomyosin association (Margossian et al., 1975). This inhibition prevails also in the presence of MgATP. Actin-activated ATPase of HMM is sensitive to the presence of Ca^{2+} provided the ionic strength of the assay system is sufficient to weaken the actin–myosin affinity. The maximum turnover rate of acto-HMM does not depend on Ca^{2+}, but the apparent dissociation constant of actin from HMM increases with the binding of this cation. Inhibition of actomyosin activity by Ca^{2+} has been previously noted by Bremel & Weber (1975) and Pemrick (1976). Our results indicate that these observations are related to the Ca^{2+} inhibition of actomyosin association, which may also account for the Ca^{2+} sensitivity of cardiac myosin (Malhotra et al., 1979). The physiological significance of the reduced binding of actin to myosin in the presence of Ca^{2+} is not clear at the present. The magnitude of the observed effect cannot easily be extrapolated to physiological conditions, and reliable actomyosin activity measurements cannot be obtained in solutions of increasing ionic strength. Thus, one must be cautious in interpreting the Ca^{2+} sensitivity in vertebrate myosin. Without doubt, the binding of actin to myosin can be reduced or increased due to the events occurring on the Nbs_2 light chains (Pemrick, 1977). However, ultimately, their regulatory function may be questioned because of the slow rate of Ca^{2+} binding (Bagshaw & Reed, 1977).

A different and new aspect of Nbs_2 light chain function should be considered in view of the phosphorylation studies of Bárány et al. (1979). These authors have found that the light chains of frog myosin are phosphorylated and dephosphorylated during the contraction of frog muscle. Phosphorylation of the light chains may considerably alter electrostatic interactions surrounding the myosin cross bridges, triggering their release from the filament (Bárány et al., 1979). Also noteworthy is the observation that the phosphorylated Nbs_2 light chains bind Ca^{2+} with higher affinity than their nonphosphorylated counterparts (Alexis & Gratzer, 1978). Future work will evaluate the significance of light chain phosphorylation to the cross bridge and Nbs_2 light chains action.

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