Effect of Calcium on Synthetic Myosin Minifilaments†

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ABSTRACT: The effect of Ca^{2+} on the structural properties of myosin filaments has been examined by employing myosin minifilaments as a model system. Paired sedimentation studies of myosin minifilaments at pH 8.0 reveal only a minor increase in their sedimentation coefficient (0.7 ± 0.3%) upon binding of Ca^{2+} (pCa = 4.5). This increase and the larger change observed at pH 7.0 (<3.7%) are attributed to protein aggregation. Light scattering measurements indicate that both Ca^{2+} and Mg^{2+} promote protein association in solutions of myosin minifilaments at pH 7.0 and 8.0 and in myosin filaments at pH 8.0. This association reaction is nonspecific and does not level off with increasing concentrations of divalent cations. Nevertheless, low concentrations of Ca^{2+} and Mg^{2+} have a rather limited effect on myosin polymerization. It is suggested that the binding of Ca^{2+} to the myosin light chains affects the association equilibrium in minifilaments and apparently does not alter other structural properties of these particles.

The regulatory function of the calcium-binding subunits (LC-2)[1] in myosin from invertebrate and smooth muscles appears to be well established (Kendrick-Jones & Scholey, 1981). Their action is mediated either by direct binding of Ca^{2+} (Lehman & Szent-Gyorgyi, 1975) or by their phosphorylation (Chacko et al., 1977; Adelstein, 1980). However, in vertebrate striated muscle the functional role of the LC-2 light chains remains unclear. In vitro tests have not detected any effect of Ca^{2+} and phosphorylation (Morgan et al., 1976) on the rate of ATP hydrolysis by unregulated actomyosin, though some effects have been noted in the regulated system (Pemrick, 1980). Consequently, the interest in the LC-2 light chains of vertebrate striated muscle has centered around their possible structural role in regulating cross-bridge movement.

The two most important examples of Ca^{2+}-induced structural changes in myosin have been provided by proteolytic digestion studies (Weeds & Pope, 1977) and hydrodynamic experiments with myosin filaments (Morimoto & Harrington, 1974). Other in vitro investigations primarily focused on the local changes in the light chains themselves (Werber & Oplatka, 1974; Alexis & Gratzer, 1978). Weeds & Pope (1977) and Bagshaw (1977) have shown that binding of divalent cations to LC-2 subunits in filamentous myosin results in protection of the S-1/S-2 junction from chymotryptic attack and promotes cleavage at the HMM/LMM junction. Since both Ca^{2+} and Mg^{2+} and even high concentrations of monovalent salts (Mrakovčič et al., 1979; Oda et al., 1980; Kardami & Gratzer, 1982) produce this striking effect, it is most probably unrelated to the function of the LC-2 chains. In fact, it appears now that the Ca^{2+}- and Mg^{2+}-induced transitions in the proteolysis of myosin can be explained without evoking structural changes in the HMM/LMM hinge (Oda et al., 1980; Borejdo & Werber, 1982).

Potentially more relevant to a putative structural function of the light chains are the observations that the sedimentation coefficient and viscosity of myosin filaments change by about 3% upon binding of Ca^{2+} (Morimoto & Harrington, 1974). These original hydrodynamic experiments of Morimoto & Harrington (1974) provide the only quantitative measurement of such changes under in vitro conditions. In principle, the Ca^{2+}-induced changes in the sedimentation and viscosity of myosin filaments could be caused by transitions in cross-bridge orientation. However, such a tempting interpretation is not supported by other studies. Neither fluorescence depolarization measurements (Mendelson & Cheung, 1976) nor cross-linking experiments (Sutoh & Harrington, 1977) can detect any Ca^{2+} effect on the disposition of myosin heads. Alternative explanations of the hydrodynamic experiments could involve other conformational transitions, swelling of the filaments, or perhaps even changes in the state of the protein's aggregation (Morimoto & Harrington, 1974). Because of the size and the complexity of the filament structure, and most of all the small magnitude of the Ca^{2+}-induced effect, definitive resolution of these questions has been hardly feasible. However, the recent description of myosin minifilaments and their properties (Reisler et al., 1980; Oriol-Audit et al., 1981) opened an obvious route for such studies.

The aim of this work was to reexamine the effect of Ca^{2+} on the hydrodynamic properties of assembled myosin, employing myosin minifilaments at pH 7.0 and 8.0. Due to their relatively small size and homogeneity the minifilaments are particularly suitable for conformational studies. At pH 8.0 we do not detect any significant Ca^{2+} effect on the sedimentation coefficient of myosin minifilaments, whereas at pH 7.0, the small increase in this parameter is attributed to a Ca^{2+}-induced aggregation process.

Materials and Methods

Distilled water and analytical-grade reagents were used in all experiments. ATP, α-chymotrypsin, and phenylmethanesulfonyl fluoride were obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation of Proteins. Rabbit myosin was prepared and stored as described by Godfrey & Harrington (1970). Solutions of synthetic myosin minifilaments in 10 mM Tris–citrate buffer at pH 8.0 were prepared as described previously (Reisler et al., 1980). The pH 7.0 minifilaments were obtained by dialyzing the pH 8.0 minifilaments against 10 mM Tris–citrate at pH 7.0. Prior to their use all solutions were centrifuged at 27000*g* for 20 min. Synthetic myosin filaments were prepared by dialyzing myosin (in 0.5 M KCl–10 mM sodium phosphate at pH 7.0) against 0.1 M KCl–10 mM Tris-HCl

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[1] Abbreviations: HMM, heavy meromyosin; LMM, light meromyosin; LC-2, Ca^{2+} binding 19000 molecular weight subunit of myosin; S-1, subfragment 1; S-2, subfragment 2; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate.

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at pH 8.0 and then against 0.1 M KCl–40 mM imidazole at pH 7.0. The concentration of minifilaments and filaments was determined by assuming an extinction coefficient of E_{280} = 5.5 cm^{-1} m (Godfrey & Harrington, 1970). The polymeric myosin was dissociated in 0.5 M KCl prior to absorption measurements.

Determination of Ca^{2+}. The concentration of free Ca^{2+} in the presence of 10 mM Tris–citrate buffer was measured by using a Ca^{2+}-selective electrode (Radiometer F2112). The electrode was calibrated by using Ca^{2+}-EGTA solutions in 40 mM Tris-HCl (pH 8.0), and 40 mM Bis-Tris (pH 7.0). Buffer solutions were treated with Chelex prior to addition of standard Ca^{2+} solution (Radiometer) and EGTA. The calibration curves were linear over the range of 3×10^{-6}–2×10^{-3} M Ca^{2+}.

Light Scattering Measurements. The intensity of scattered light from solutions of myosin minifilaments and filaments (1 mg/mL) was measured at an angle of 90° with an Aminco fluorescence spectrophotometer. The measurements were carried out at room temperature, at a wavelength of 540 nm, and the results were expressed in arbitrary units. Protein solutions were clarified by centrifugation (at 27000*g* for 30 min) prior to scattering measurements.

Chymotryptic Digestions. Myosin minifilaments (3 mg/mL) in 10 mM Tris–citrate, at pH 7.0 and 8.0, were digested with α-chymotrypsin (0.05 mg/mL), at room temperature, in the presence and absence of added Ca^{2+}. The proteolysis was terminated by adding phenylmethanesulfonyl fluoride (1 mM). The digested myosin samples were then denatured and examined on $NaDodSO_4$–polyacrylamide gels as described previously (Mrakovčič et al., 1979).

Sedimentation Experiments. Paired sedimentation velocity experiments (Morimoto & Harrington, 1974) were carried out at rotor speeds of (15–30) $\times 10^3$ rpm and temperatures close to 20 °C in a Spinco Model E analytical ultracentrifuge. The experiments involved running in the same rotor two 12-mm path length cells, one wedge cell and one regular cell. This method allows precise determination of small Ca^{2+}-induced changes in the sedimentation coefficient of myosin filaments (Morimoto & Harrington, 1974). In each experiment one cell contained minifilament solution (3 mg/mL, 10 mM Tris–citrate pH 8.0 or 7.0) in the presence of Ca^{2+}, and the other cell had an identical solution without added Ca^{2+}. Addition of Ca^{2+} and preparation of solutions for sedimentation experiments was done as described by Morimoto & Harrington (1974). Particular care was taken to match the volume of liquids in both cells. Runs which showed slight mismatch in solution volumes (as indicated by the initial boundary position) were rejected to avoid corrections for radial dilution and *s.* vs. *c* dependence. Control paired sedimentation runs of the same minifilament solution (in both cells) revealed no measurable difference in their sedimentation coefficients (less than 0.5%). All sedimentation runs were monitored by employing the schlieren optical system.

Results

Earlier work has shown that the high-affinity binding sites for Ca^{2+} on myosin are fully saturated at pCa = 5 (Weeds & Pope, 1977; Kardami et al., 1980; Morimoto & Harrington, 1974). Accordingly, the changes in hydrodynamic properties (Morimoto & Harrington, 1974) and proteolytic digestion of myosin filaments (Weeds & Pope, 1977) occur between pCa = 7 and pCa = 5. In most cases, the free Ca^{2+} concentration is controlled by an appropriate choice of total EGTA and Ca^{2+} concentrations. However, in myosin minifilaments which are routinely prepared in 10 mM Tris–citrate buffer at pH 8.0 (Reisler et al., 1980), the divalent cations are chelated by citrate ions. Using a Ca^{2+}-selective electrode, we have determined that in the absence of added Ca^{2+} the level of free Ca^{2+} falls in this solvent below 10^{-6} M. This result was confirmed by chymotryptic digestion experiments on myosin minifilaments. They yielded the same products, subfragment 1 and rod, for reactions carried out in the presence and absence of 1 mM EDTA (Figure 1). Consequently, EDTA was omitted from most of our experiments.

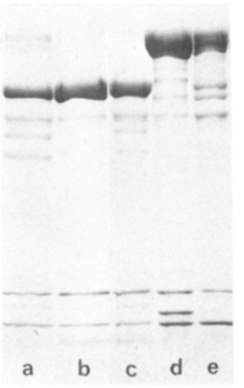

FIGURE 1: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of water-soluble fragments produced by chymotryptic digestion of myosin minifilaments. Fragments were run on composite 10 and 15% polyacrylamide gels. All digestions were carried out at room temperature, with 0.05 mg/mL chymotrypsin. (a) Myosin minifilaments (3 mg/mL) digested for 5 min in 10 mM Tris–citrate, pH 7.0; (b) standard S-1 prepared by chymotryptic digestion of myosin filaments in 0.12 M NaCl–10 mM sodium phosphate (pH 7.0) and 1 mM EDTA (Weeds & Pope, 1877); (c) as in (a) except for addition of 1 mM EDTA; (d) standard HMM prepared as described by Weeds & Pope (1977); (e) myosin minifilaments (3 mg/mL) digested for 5 min in 10 mM Tris citrate (pH 8.0) and at pCa = 4.5.

The effect of Ca^{2+} on the sedimentation of myosin minifilaments was measured under Ca^{2+} saturation conditions, at pCa = 4.5. The amount of Ca^{2+} (1 mM) needed in order to give this level of free cation in 10 mM Tris–citrate (pH 8.0) was determined by employing the Ca^{2+}-selective electrode. Chymotryptic digestions of minifilaments confirmed that the LC-2 light chains were indeed saturated by Ca^{2+} (Figure 1). As expected in such case, the major product of myosin proteolysis was HMM (Weeds & Pope, 1977). In view of this result, and because of detectable cation-induced aggregation of myosin at higher Ca^{2+} concentrations, the paired sedimentation experiments were confined to pCa = 4.5.

Measurements of light scattered (at 90°) from solutions of minifilaments revealed small but consistent increase in their turbidity with increasing Ca^{2+} concentration (Figure 2). At pCa = 4.5 (pH 8.0) this increase was hardly notable and amounted to about 0.7 ± 0.5% of the original scattering in the absence of Ca^{2+}. However, the aggregation of myosin minifilaments at pH 7.0 (in 10 mM Tris–citrate) was more pronounced under similar conditions, yielding about a 3–4% scattering increase at pCa = 4.5.

Effect of Ca^{2+} on the Sedimentation Coefficient of Synthetic Myosin Minifilaments. Figure 3 shows sedimentation boundary profiles for myosin minifilaments (3 mg/mL) in the absence and presence of Ca^{2+}. The hypersharp barlike appearance of these patterns, which persists throughout the entire run, is particularly advantageous in the paired sedimentation experiments. It allows satisfactory resolution of two boundaries for solutions which differ by less than 1% in their sedimentation coefficients. Consequently, the solution volumes in the two cells can be carefully matched, thus alleviating the requirement for radial dilution related corrections. The paired sedimentation runs were carried out with seven different myosin

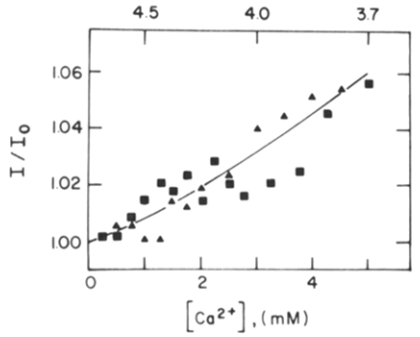

FIGURE 2: Normalized intensity of scattered light from solutions of myosin minifilaments (1 mg/mL) at pH 8.0 as a function of added Ca^{2+}. The scattering was measured at 90°, at 540 nm, and was normalized to the intensity of scattered light in the absence of Ca^{2+} (I_0). The actual amounts of free Ca^{2+} (pCa) present in a 10 mM Tris–citrate solvent (pH 8.0) were determined by employing a Ca^{2+}-selective electrode (Materials & Methods), and are given in the upper scale. The different symbols represent two separate sets of experiments.

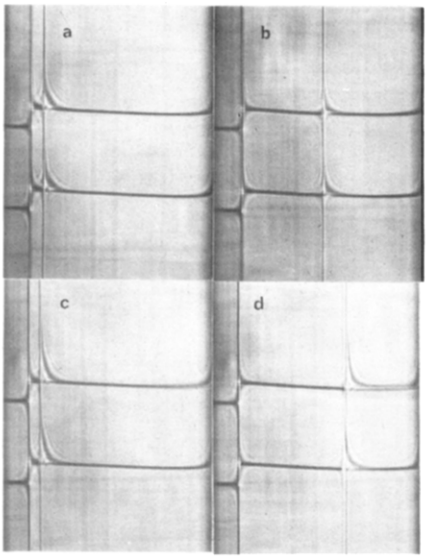

FIGURE 3: Paired sedimentation velocity experiments on myosin minifilaments in 10 mM Tris–citrate at pH 8.0 (a, b) and 7.0 (c, d). Temperature was 20–21 °C, protein concentration was 3 mg/mL, rotor speed was 31 000 rpm, and bar angle was 60°. Lower patterns, minifilaments in the absence of Ca^{2+}; upper patterns, minifilaments and Ca^{2+} (pCa 4.5). Pictures a and b were taken 10 and 74 min after reaching the final speed; frames c and d were taken 5 and 69 min after reaching the final rotor speed. The respective sedimentation coefficients, $s_{20,w}$, of the pH 8.0 (a, b) and 7.0 (c, d) minifilaments in the absence of Ca^{2+} were 16.97 and 23.18 S. Ca^{2+} induced no measurable increase in the sedimentation coefficient of minifilaments at pH 8.0 and a 2.4% increase at pH 7.0.

minifilament preparations (at pH 8.0). Their sedimentation coefficients, $s_{20,w}$ at 3 mg/mL protein concentration, varied between 16 and 17 S, in agreement with our previous determinations (Reisler et al., 1980). The average Ca^{2+}-induced increase in the sedimentation coefficient of these minifilaments, $[(s_{Ca} - s_0)/s_0] \times 100$, was 0.7 ± 0.3%. The individual results varied between zero Ca^{2+} effect (Figure 3a,b) and 1.1% change. However, even this small and hardly significant effect resulted probably from the Ca^{2+}-induced aggregation of minifilaments. Such possibility is suggested by light scattering measurements, which detect protein aggregation (Figure 2), and by the experiments carried out with the pH 7.0 minifilaments.

Myosin minifilaments at pH 7.0 (in 10 mM Tris–citrate) are larger and somewhat less homogeneous than their pH 8.0

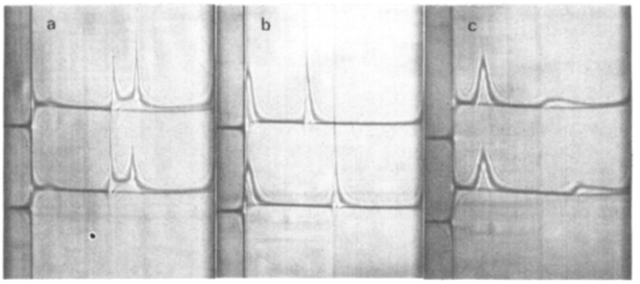

FIGURE 4: Effect of Ca^{2+} on myosin assembly system. (a) Myosin minifilaments (3 mg/mL) in 10 mM Tris–citrate (pH 8.0) 2 h after addition of 80 mM KCL. Lower pattern, in the absence of Ca^{2+}; upper pattern, in the presence of Ca^{2+} (pCa = 4.5). The faster peak corresponds to myosin filaments, the slower peak represents the minifilaments, and the slowest one corresponds to dissociated myosin (Reisler et al., 1982). Rotor speed was 31 000 rpm. (b and c) Myosin in 0.18 M KCl–0.02 M Tris-HCl, pH 8.0, was initially sedimented at 14 000 rpm (b) and then the rotor speed was raised to 44 000 rpm (c). Upper patterns, in the absence of Ca^{2+} (1 mM EDTA); lower patterns, in the presence of 0.1 mM Ca.

counterparts. Nevertheless, they remain superior in terms of size homogeneity to any other filamentous forms of myosin at pH 7.0. In the absence of Ca^{2+}, the pH 7.0 minifilaments (3 mg/mL) had a sedimentation coefficient, $s_{20,w}$, between 23 and 24 S. At zero protein concentration, they extrapolated to $s_{20,w}$ = 38 S, compared to $s_{20,w}$ = 33 S for the pH 8.0 minifilaments. As observed in paired sedimentation runs, Ca^{2+} (pCa = 4.5) induced between a 2.4 and 3.7% increase in the sedimentation coefficient of the pH 7.0 minifilaments (Figure 3c,d). Typically, in these experiments, addition of Ca^{2+} to minifilaments resulted in a slight elevation of the leading part of the schlieren boundary over the base line, which indicated the presence of aggregated protein. This finding, in conjunction with the Ca^{2+}-induced increase in the light scattering of the pH 7.0 minifilaments (3–4% increase at pCa = 4.5), suggested that the observed changes in the sedimentation coefficient of myosin minifilaments were related to their aggregation. Further support for this contention was provided by the fact that the increase in the turbidity of minifilament solutions could be also induced by Mg^{2+} ions and did not show any saturation behavior with increasing concentrations of either Ca^{2+} or Mg^{2+}.

Effect of Ca^{2+} on the Association Reaction of Myosin. In order to further explore the nature of Ca^{2+}-induced changes in myosin minifilaments, we have examined the effect of low concentrations of this cation on the myosin polymerization equilibrium. Recently, we showed that addition of KCl to synthetic myosin minifilaments (at pH 8.0) induced their growth into larger filaments (Reisler et al., 1982). As described in that work, 2 h after addition of 80 mM KCl the assembly system consisted of minifilament and filament particles and a small amount of dissociated myosin. In Figure 4a we compare such assembly system in the absence and presence of Ca^{2+} (pCa = 4.5). In the latter case a larger fraction of protein migrates under the fast sedimenting peak, indicating a clear shift of the polymerization reaction in favor of filament formation. Similar Ca^{2+} effects could be also detected under conventional myosin assembly conditions, i.e., in the absence of minifilaments. When 0.1 mM Ca^{2+} was added to a monomer–filament solution in 0.18 M KCl and 20 mM Tris-HCl (pH 8.0), it increased the fraction of polymerized myosin (Figure 4b,c). As documented by Josephs & Harrington (1966), under these solvent conditions a significant fraction of myosin exists in a dissociated form, thus facilitating detection of any changes in the monomer–polymer equilibrium.

In the present case, Ca^{2+} affected both the size of the filaments and the monomer–filament distribution. The larger size of filaments in the presence of Ca^{2+} was indicated by their faster sedimentation rate. For determination of the amount of myosin monomer in equilibrium with the filaments, the rotor speed was raised after the polymer boundaries were well resolved from the dissociated myosin, and the areas under the monomer peaks were compared (Figure 4b,c). In the presence of Ca^{2+}, the amount of dissociated myosin was about 15% smaller than in the absence of Ca^{2+}. In contrast to these rather limited changes in monomer–polymer equilibrium at low Ca^{2+} concentrations, a significant aggregation of myosin was induced at higher levels of divalent cations. Thus, for example, the turbidity of myosin filaments (OD_{310}) in 0.1 M KCl and 0.025 M Tris-HCl at pH 8.0 (3 mg/mL) increased by about 12–15% in the presence of 1 mM Ca^{2+} or Mg^{2+}. However, synthetic myosin filaments prepared at pH 7.0 did not reveal any Ca^{2+}-induced aggregation in light scattering measurements.

Discussion

Our choice of myosin minifilaments for examining the structural consequences of Ca^{2+} binding to assembled myosin is based on the following reasons: (i) Myosin minifilaments constitute a highly homogeneous system of small bipolar filaments which resemble the central part of synthetic myosin filaments (Reisler et al., 1980). Because of the small size and homogeneity of minifilaments any changes in their hydrodynamic properties may be easily measured and interpreted. (ii) In a previous study we have shown that myosin minifilaments and filaments indeed have common structural properties, among them their interaction with nucleotides, pyrophosphate, and other ligands which destabilize the organized myosin polymer (Oriol-Audit et al., 1981). (iii) Binding of Ca^{2+} to myosin minifilaments affects their chymotryptic digestion in the same manner as normally observed for myosin filaments; i.e., divalent cations block the degradation of the LC-2 light chains and inhibit the formation of subfragment 1. (iv) Preliminary studies indicate also that increasing the pH of myosin minifilaments from 7.0 to 8.0 results in a decrease in the rate of subfragment 2 cross-linking and an increase in the proteolytic susceptibility of the HMM/LMM hinge (D. Applegate and E. Reisler, unpublished results), in analogy to the behavior of myosin filaments, myofibrils, and fibers (Ueno & Harrington, 1981). In view of these considerations it might be expected that if Ca^{2+} affects the structural properties of myosin cross-bridges and filaments, then such effects should be expressed and detected at the level of myosin minifilaments.

The paired sedimentation experiments on myosin minifilaments at pH 8.0 reveal only a minor Ca^{2+}-induced increase ($0.7 \pm 0.3\%$) in their sedimentation coefficient. As shown by light scattering measurements this increase arises probably from Ca^{2+}-mediated changes in the protein's aggregation. At pH 7.0 the sedimentation changes are larger (2.4–3.7%), but again they can be attributed to protein aggregation. More specifically, the faster sedimentation rate of minifilaments in the presence of Ca^{2+} could be due to their reduced concentration resulting from preferential depletion of a small fraction of aggregated myosin (Figure 3). Since divalent cations clearly promote an association reaction in solutions of minifilaments, it should be remembered that the reported increases in their sedimentation coefficients are measured at low Ca^{2+} concentrations (pCa = 4.5). Under such conditions, when Ca^{2+} binds primarily to the LC-2 light chains, the induced myosin polymerization is rather limited and perhaps mainly expressed as a shift in a monomer–polymer equilibrium in the myosin assembly system (Figure 4). It is unlikely that this stimulation of the polymerization reaction is due to nonspecific ionic effects such as chelation of citrate ions by Ca^{2+} or an increase in the ionic strength of the monomer–filament solution (in 0.18 M KCl, pH 8.0). Control experiments show that at lower citrate ion concentrations (<10 mM) the minifilaments decrease in size. Similarly, an increase in the ionic strength of the monomer–filament solution (in 0.18 M KCl, pH 8.0) results in a partial dissociation of the polymeric species (Josephs & Harrington, 1966). Thus, it appears that at low Ca^{2+} concentrations the modulation of protein–protein interactions is linked to the state and conformation of the LC-2 light chains. Such an explanation is not without precedent, since it has been observed that slight proteolysis of these chains in rat skeletal myosin prevents the formation of long regular filaments (Pinset-Härström & Whalen, 1979), whereas phosphorylation of LC-2 subunits in smooth muscle myosin stabilizes the filamentous structures (Suzuki et al., 1978).

The alternative possibility that nonspecific binding of Ca^{2+} to low-affinity sites on myosin ($K_d = 10^{-3}$ M; Borejdo & Werber, 1982) is responsible for protein aggregation at pCa = 4.5 seems unlikely but cannot be excluded. We calculate that under our experimental conditions at most 2.3% myosin has weakly bound Ca^{2+} (at pCa = 4.5). Although this appears to be consistent with the small protein aggregation detected in light scattering and sedimentation experiments, it could hardly account for the observed significant shifts in the polymerization equilibrium of myosin (Figure 4). These changes, if related to the presence of a minor fraction of myosin with weakly bound Ca^{2+}, might be expected to be of a comparatively small magnitude.

We note that both the limited (at low Ca^{2+} concentrations) and the extensive (at $[M^{2+}] = 0.5$ mM) changes in the myosin association reaction are observed for myosin minifilaments at pH 7.0 and 8.0 and for myosin filaments at pH 8.0. However, in agreement with Morimoto & Harrington (1974), our light scattering measurements of myosin filaments at pH 7.0 do not reveal any aggregation of these particles in the presence of Ca^{2+} (up to 1 mM M^{2+}). The different behavior of the pH 7.0 filaments may be attributed to the fact that these particles are much larger than the minifilaments and the pH 8.0 filaments and probably do not undergo any significant length redistribution.

The nature of Ca^{2+}-specific changes in the hydrodynamic properties of myosin filaments around neutral pH (Morimoto & Harrington, 1974) remains unclear. One possibility is that these changes are conditioned upon the presence of nucleotides. We have not included MgADP or MgATP in our experiments because of their effect on minifilament (Oriol-Audit et al., 1981) and filament structures (Harrington & Himmelfarb, 1972; Pinset-Härström & Truffy, 1979) and the consequent ambiguities in the interpretation of results. Our present results demonstrate that binding of divalent cations to myosin may affect protein–protein interactions in assembled protein. Yet, myosin minifilaments which appear fully competent in terms of conformational transitions do not show any Ca^{2+}-induced structural changes except for trivial and minor aggregation process. Thus, in line with other evidence (Sutoh & Harrington, 1977; Mendelson & Cheung, 1976), we conclude on the basis of our sedimentation studies on myosin minifilaments that low concentrations of divalent cations do not affect the disposition of cross bridges in the organized myosin structure.

Acknowledgments

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Comparison of the Actin Binding and Filament Formation Properties of Phosphorylated and Dephosphorylated *Acanthamoeba* Myosin II†

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ABSTRACT: Dephosphorylated (less than 0.5 mol of phosphate/mol of heavy chain) and maximally phosphorylated (3 mol of phosphate/mol of heavy chain) *Acanthamoeba* myosins II were compared. Actin-activated Mg^{2+}-ATPase activity was highest for dephosphorylated myosin II at 4-4.5 mM Mg^{2+} and at 7 mM Mg^{2+} for phosphorylated myosin II. At all concentrations of Mg^{2+}, activity was higher for the dephosphorylated enzyme. Assays of the actomyosin ATPase activities as a function of F-actin concentration showed that this higher enzymatic activity is attributable to the higher V_{max} value of the dephosphorylated form of myosin II; in fact, the K_{ATPase} value was higher for the more active, dephosphorylated form than for the less active, phosphorylated form. Both forms of myosin II bound essentially completely to F-actin in the presence of ATP, but the dephosphorylated form of the enzyme may have a greater affinity for F-actin, although insufficient to account for the higher actin-activated ATPase activity of dephosphorylated myosin II. Both dephosphorylated myosin II and phosphorylated myosin II formed bipolar filaments in 10 mM $MgCl_2$-20 mM KCl, but the filaments of the dephosphorylated form were larger and less readily dissociated by approximately physiological concentrations of MgATP. The dephosphorylated enzyme formed filaments, in the absence of Mg^{2+}, at KCl concentrations as high as 200 mM while phosphorylated myosin II was fully soluble at 200 mM KCl and 85% soluble at 25 mM KCl. Therefore, dephosphorylation of the heavy chains of *Acanthamoeba* myosin II increases its self-association, possibly its binding to F-actin, and the V_{max} of its actin-activated ATPase activity. There is no evidence that these three effects of dephosphorylation of myosin II are causally related but it seems likely that all are the result of structural changes in the myosin filaments.

Acanthamoeba myosin II contains a pair of heavy chains of about 185 000 daltons and two pairs of light chains of about 17 500 and 17 000 daltons (Maruta & Korn, 1977; Pollard et al., 1978; Collins & Korn, 1980, 1981). Collins & Korn (1980) showed that myosin II contains about 1.2-1.5 mol of phosphate/heavy chain when isolated from cells by their procedure (Collins & Korn, 1981) and that it contains about 2 mol of phosphate/heavy chain when isolated by the procedure of Pollard et al. (1978). Myosin II can be maximally phosphorylated by a partially purified kinase to the extent of 3 mol of phosphate/heavy chain, all esterified to serine residues (Cote et al., 1981). Phosphorylation of the light chains of

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