Influence of myosin heavy chains on the Ca$^{2+}$-binding properties of light chain, LC$_2$

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The association of myosin light chains with heavy chains, i.e. the intact oligomeric structure, profoundly affects the Ca$^{2+}$-binding properties of the light chains. The Ca$^{2+}$-binding affinity of the light chains is more than two magnitudes higher in the presence of heavy chains than in its absence. Modification of the reactive SH$_2$ thiol of myosin results in an alteration in the conformation of heavy chains of the molecule that influences the Ca$^{2+}$-binding properties of light chains and generation of tension. When the SH$_2$ moiety is blocked with N-ethylmaleimide the influence of the heavy chains on the Ca$^{2+}$-binding properties of light chain LC$_2$ is lost; under these conditions the Ca$^{2+}$-binding affinity value of SH$_2$-N-ethylmaleimide-blocked myosin (3.3 x 10$^4$ M$^{-1}$) decreases to nearly that expressed with the dissociated light chain LC$_2$ (0.7 x 10$^4$ M$^{-1}$). Conversely, the presence of actin, nucleotides or modification of either the reactive lysyl residue or SH$_2$ thiol does not affect Ca$^{2+}$ binding. The native secondary and tertiary structure of myosin seem to be required for Ca$^{2+}$ binding; binding does not occur in the presence of 6M-urea with either native myosin or the dissociated light chains. With SH$_2$-N-ethylmaleimide-blocked myosin normal Ca$^{2+}$- and (Mg$^{2+}$ + actin)-stimulated ATPase activities are expressed; however, there is a loss in K$^+$-stimulated ATPase activity and the synthetic actomyosin threads of such myosin express no isometric tension. There are also variances in the binding of Ca$^{2+}$ with alterations in pH values. In the absence of Ca$^{2+}$/EGTA buffer the biphasic Ca$^{2+}$-binding affinity of myosin is twice as high at pH 7.4 (site one: 1.2 x 10$^6$ M$^{-1}$ and site two: 0.4 x 10$^6$ M$^{-1}$) as compared with values obtained at pH 6.5 (site one: 0.64 x 10$^6$ M$^{-1}$ and site two: 0.2 x 10$^6$ M$^{-1}$). The Ca$^{2+}$-binding affinity of light chain LC$_2$ and S$_i$, where the (S-1)–(S-2) junction was absent, were not influenced by changes in pH values. Both expressed a low Ca$^{2+}$-binding affinity, approx. 0.7 x 10$^4$ M$^{-1}$, whereas heavy meromyosin, where both (S-1) and (S-2) myosin subfragments were present, expressed a Ca$^{2+}$-binding affinity value similar to that of native myosin, but was not biphasic. However, it is important to point out that in preparation of $S_i$ myosin subfragment light chain LC$_2$ was lost and thus was added back to the purified $S_i$ fraction. Light chain LC$_2$ was not, however, added to the heavy meromyosin fraction because it was not lost during preparation of the heavy meromyosin subfragment. In conclusion, it appears that the (S-1)–(S-2) junction is needed for the positioning of light chain LC$_2$ and thus influences its essential conformation for Ca$^{2+}$ binding.

Abbreviations used: HC, heavy chain; LC$_i$, light chain-$i$; LC$_2$, Ca$^{2+}$-binding light chain-$2$; LC$_3$ (second alkali light chain), light chain-$3$; S$_i$, myosin subfragment head; SDS, sodium dodecyl sulphate; Tes, 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulfonic acid.

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Fragmentation of heavy meromyosin results in three S-1 peptides (Balint et al., 1978; Cardinaud, 1979; Mornet et al., 1979). The 27 K-mol.wt. tryptic peptide includes the N-terminal (Cardinaud, 1979), the reactive lysine residue critical for complete myosin ATPase activity (Takashi et al., 1980) and the nucleotide binding site(s) (Yamamoto & Sekine, 1980; Szilagyi et al., 1979); adjacent to this is the 50 K-mol.wt. peptide containing the actin-binding site (Mornet et al., 1979) and then a 20 K-mol.wt. fragment containing the SH1 and SH2 reactive thiol groups (Balint et al., 1978; Cardinaud, 1979). Adjacent to the (S-1) 20 K-mol.wt. fragment is an (S-2) (Balint et al., 1975) 40 K-mol.wt. peptide (Cardinaud, 1979). The (S-1)—(S-2) junction, i.e. the chymotryptic-EDTA-susceptible region (Kuwaryama & Yagi, 1979), appears to be located in this area (Cardinaud, 1979). The region between myosin subfragments S-1 and S-2 contains the universal joint (swivel region) that allows the myosin head to freely pivot around a flexible joint (Mendelson & Cheung, 1976). Light chain LC1 interacts with the (S-1)—(S-2) junction and the 20 K-mol.wt. peptide containing the SH1 and SH2 thiol groups (Srivastava et al., 1980; Cardinaud, 1979). The (S-1)—(S-2) junction may be necessary for the positioning of light chain LC2 in rabbit skeletal muscle myosin, as (S-1)-containing LC2 is unregulated in scallop myosin whereas single-headed myosin containing Ca2+ and LC2 is regulated (Stafford et al., 1979). The Ca2+-binding light chain of rabbit skeletal muscle myosin can be substituted for that of scallop (Kendrick-Jones et al., 1970) to restore Ca2+-sensitivity to desensitized scallop myosin.

Factors that cause dissociation of light chain LC2 may also cause alterations in the swivel region of myosin. Light chain LC2 is more firmly attached to myosin in the presence of Ca2+ (Szent-Gyorgyi, 1975; Kasman & Kadol, 1977). When in association with the heavy chains the binding of Ca2+ is weaker at pH 6.5 as compared with pH 7.4 (Wikman-Coffelt, 1980; Muhlrad et al., 1962; Werber, 1978). H+ may induce conformational changes in the swivel region of the heavy chains (Mendelson & Cheung, 1976; Chiano & Harrington, 1979), whereas pH has no influence on the conformation of the dissociated light chains (Werber, 1978; Stafford & Szent-Gyorgyi, 1978). The light chains are not denatured with dissociation because reassociation again promotes a high Ca2+-binding affinity (Wikman-Coffelt et al., 1979; Okamoto & Yagi, 1977). Likewise, increases in temperature that induce light chain dissociation in the presence of chelating agents (Stafford et al., 1979; Wikman-Coffelt et al., 1979; Higuchi et al., 1978) also promote conformational changes in myosin heavy chains (Mendelson & Cheung, 1976; Chiano & Harrington, 1979; Ishigami & Morita, 1977), but not the light chains (Chantler & Szent-Gyorgyi, 1978; Stafford & Szent-Gyorgyi, 1978).

**Experimental**

Rabbit skeletal muscle myosin was purified by methods described earlier (Fabian et al., 1977) and actin was prepared from rabbit skeletal muscle by the method of Spudich & Watt (1971). Purification of S-1 was as described by Weeds & Taylor (1975) and heavy meromyosin as described by Chiano & Harrington (1979).

Light chains were prepared with 8 M-urea as described in earlier reports (Long et al., 1977), except that the light chains were concentrated by 80% satd.-NH4)2SO4 precipitation instead of freezedrying. Light chains were stored at 4°C and used within 1 week after preparation.

For gel electrophoresis the 5–20% polyacrylamide-gradient slabs were prepared as described in earlier reports (Long et al., 1977); this type of high resolution electrophoresis contained SDS and thus allowed for identification of peptides by molecular weight. Use of polyacrylamide gels containing urea was by the method of Ritz-Gold et al. (1980), which was a modification of that by Pires & Perry (1977), and allowed for identification of myosin light chain LC2 phosphorylation.

Ca2+-activated ATPase activity was measured in a medium containing 4 mM-ATP/0.5 M-KCl/10 mM-CaCl2/0.02 M-Tris/HC1 (pH 7.6) at 30°C. (Actin+Mg2+)-activated myosin ATPase activity was measured in a medium containing 0.05 M-Tes (pH 7.0)/0.05 M-KCl/4 mM-MgCl2/1 mM-ATP with 0.1 mg of myosin/ml and 0.1 mg of actin/ml at 25°C. K+/EDTA-activated ATPase activity of myosin was measured in a mixture containing 0.05 M-Tris/HCl (pH 7.6)/5 mM-EDTA/0.5 M-KCl/5 mM-ATP at 30°C. The time was chosen so that <15% of ATP was hydrolysed during the course of the reaction. P1 was estimated by the method of Muhrlen & Eisenberg (1976).

The SH2 thiol of myosin was selectively blocked as described by Reiser et al. (1974), with slight modifications. Myosin (0.2% in 0.5 M-KCl/0.01 M-Tris/histidine, pH 8.3) was incubated with a 4-fold excess of fluorodinitrobenzene for 30 min at 5°C and then precipitated with 45% satd.-NH4)2SO4; (NH4)2SO4 precipitation was used instead of lowering the ionic strength because of the difficulty of precipitating myosin at this higher pH. Myosin was centrifuged, the pellet washed in water, solubilized in 0.5 M-KCl/0.05 M-Tris/histidine (pH 7.9)/1 mM-MgADP and was incubated with a 4-fold excess of N-ethylmaleimide for 30 min at 5°C. The reaction was stopped with 45% satd.-NH4)2SO4. The mixture was centrifuged and the pellet washed twice in
water; myosin was then dissolved in 0.5 M-KCl/0.05 M-Tris/histidine (pH 7.5). The dinitrophenylated groups were subsequently removed by treating the protein for 45–60 min with 0.05 M-β-mercaptoethanol in 0.5 M-KCl/0.01 M-Tris/histidine (pH 8.5)/1 mM-EDTA, under nitrogen at room temperature. The protein was subsequently precipitated with 10 vol. of water, then centrifuged and solubilized in 0.5 M-KCl/0.05 M-Tris/histidine (pH 7.0) and dialysed extensively against this buffer. Control samples were similarly treated except that the incubation step with N-ethylmaleimide was omitted.

The two reactive lysine residues involved in the active site of myosin were modified with 2,4,6-trinitrobenzenesulphonate as described in earlier reports by Muhlrad et al. (1976).

Isometric tension was measured with synthetic actomyosin threads as described earlier (Srivastava et al., 1980). Threads were formed by extrusion of the concentrated solution of actomyosin through a syringe into a trough that contained buffer (20 mM-KCl/5 mM-MgCl₂/10⁻³ M-CaCl₂/20 mM-Tris, pH 7.0). The threads were removed from the trough, mounted on the tensiometer and glued to the mounting rod with a drop of acetone and Plexiglass. The tensiometer was as designed and described in detail by Crooks & Cooke (1977). The threads were then immersed in 2.5 ml of the above buffer maintained at 24°C containing 4 mM-phosphocreatine and 0.4 mg of creatine kinase/ml. The threads were allowed to equilibrate with the tensiometer in the isometric mode; ATP was added and the tension increased. After the tension reached its maximum, the tensiometer was switched momentarily into the isotonic mode and the position of the arm was recorded on an x-y recorder. The tensiometer was returned to the isometric mode and the thread was allowed to return to its initial length before subsequent velocity was recorded at a different tension.

Ca²⁺ binding was carried out in the absence of a Ca²⁺/EGTA buffer as described earlier (Wikman-Coffelt & Muhlrad, 1980) so as to avoid the disparate results of using ⁴⁰Ca along with a ⁴⁰Ca²⁺/EGTA system. Two binding sites were obtained for myosin in the absence of the metal buffer and the binding affinity was higher than if such a buffer was present (Wikman-Coffelt & Muhlrad, 1980). All buffers and water were rapidly passed through Chelex-100 immediately before use to remove bivalent cation contaminants. All treatment was continued rapidly to avoid an equilibrium of contaminants with Chelex. All containers, measuring flasks and pipettes were of a disposable plastic. Portions of myosin (7–8 mg/ml) or light chains (1 mg/ml) were added to individual bags (1 ml) and predialysed in 0.2 M-Tris/maleate (pH 7.4 or 6.5)/0.1 M-KCl/1 mM-EDTA/1 mM-dithiothreitol. The second and third dialysis contained the same except that no EDTA was present. Stock solutions were prepared as described earlier (Wikman-Coffelt & Muhlrad, 1980). Under these conditions the contaminants, Ca²⁺ and Mg²⁺, were less than 10⁻⁷M. The high buffer concentration retained a constant pH throughout the reaction. The Ca²⁺ concentration was varied from 10⁻⁷ to 10⁻⁵M for all analyses; experimental and control samples were dialysed together in the same flask as described by Holroyde et al. (1979). To determine the number of sites and affinity values of the dissociated light chains, heavy meromyosin S-1 and SH₂-N-ethylmaleimide-blocked myosin Ca²⁺ concentrations of 10⁻⁵–10⁻³M were also analysed. At this range of Ca²⁺ concentrations the two binding sites on the myosin oligomer were filled; however, all of the sites on the SH₂-N-ethylmaleimide-blocked myosin, S-1 and the light chains were not filled until the Ca²⁺ concentration was raised to 10⁻³M. The dialysis medium consisted of 0.2 M-Tris/maleate (pH 6.5 or 7.4)/0.1 M-KCl and 5 µCi of ⁴⁰Ca in a total volume of 100 ml. After a dialysis time of 48 h (4°C), with continuous shaking, samples from both inside and outside the bag were removed (0.1 ml) in duplicates, an equal volume of H₂O₂ was added and the mixture heated in closed vials for 4 h at 55°C; 10 ml of scintillation fluid (PPO) was added and the samples were analysed in a Beckman scintillation counter for assessing radioactivity. Duplicate samples of protein solution inside the bags were taken for the determination of protein. Calculations were as those described earlier (Wikman-Coffelt & Muhlrad, 1980).

Results

After SH₂ modification, myosin expressed normal Ca²⁺- and (actin + Mg²⁺)-activated ATPase activity (Table 1), similar to that described by Schaub et al. (1979) for SH₂ modification of myosin. The SH₂-N-ethylmaleimide-blocked myosin expressed no isometric tension when analysed as synthetic actomyosin threads (Fig. 1). Similarly, blocking of the SH₂ thiol of myosin resulted in a large decrease in Ca²⁺-binding properties of the light chain (see Fig. 3). The Ca²⁺-binding affinity of SH₂-N-ethylmaleimide-blocked myosin (3.3 × 10⁴ M⁻¹) (Figs. 2 and 3), the dissociated light chains (0.7 × 10⁴ M⁻¹) (Fig. 4) and S-1 (0.8 × 10⁴ M⁻¹) (Fig. 4) were considerably lower than that of native myosin oligomer (Figs. 3 and 5). The Ca²⁺-binding affinity values for the purified light chains were similar to that of earlier reports (Kuwayama & Yagi, 1979; Wikman-Coffelt, 1980; Wikman-Coffelt et al., 1979). Native myosin consistently expressed a higher Ca²⁺-binding affinity when assayed at pH 7.4 as compared with pH 6.5 (Figs. 2 and 3), similar to reports where Ca²⁺/EGTA buffer was added (Werber, 1978; Wikman-
Table 1. Activities of myosin ATPase after SH2-modification

Myosin (0.2% in 0.5 M-KCl/0.01 M-Tris/histidine, pH 8.3) was incubated with a 4-fold excess of fluoro-dinitrobenzene for 30 min at 5°C and then precipitated with 45%-satd.-{(NH4)2}SO4. After centrifugation the pellet was washed in water and solubilized with a 4-fold excess of N-ethylmaleimide for 30 min at 5°C. The reaction was stopped, the mixture centrifuged and the pellet washed twice in water. Myosin was then dissolved in 0.5 M-KCl/0.05 M-Tris/histidine (pH 7.5). After removal of the dinitrophenylated groups, the protein was precipitated with 10 vol. of water, centrifuged and solubilized in 0.5 M-KCl/0.05 M-Tris/histidine (pH 7.0) and dialysed against this buffer. Such SH2-modified myosin was then assayed for K+/EDTA- (Actin + Mg2+)- stimulated and Ca2+-stimulated ATPase activities as described in the Experimental section.

<table>
<thead>
<tr>
<th>ATPase activity</th>
<th>(μmol of P1 min⁻¹ mg⁻¹ of myosin)</th>
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<tbody>
<tr>
<td></td>
<td>K⁺-EDTA-stimulated (Actin + Mg2⁺)-</td>
</tr>
<tr>
<td>Control</td>
<td>2.90</td>
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<tr>
<td>SH2-blocked</td>
<td>0.63</td>
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Fig. 1. Force velocity curve of actomyosin threads
Threads were formed from the solution of myosin and actin (55 mg/ml) in a ratio of 1:2. Native myosin (■—■) and SH2-N-ethylmaleimide-blocked myosin, shown by the empty circle at the origin, were analysed. The process was initiated with 125 μg of ATP, similar to conditions reported earlier (Srivastava et al., 1980).

Coffelt, 1980); likewise comparable results were obtained by Muhlrad et al. (1962), where analyses were carried out in the absence of a Ca2⁺/EGTA.
Ca\textsuperscript{2+}-binding properties of light chain LC\textsubscript{2}

S-1 was cleaved from S-2 with the chymotryptic digestion procedures described here (Taylor & Weeds, 1975) and thus S-1 lacked an important (S-1)–(S-2) junction that may be required for Ca\textsuperscript{2+} binding, as in the case of cardiac myosin (Kuwanyama & Yagi, 1979). Light chains were added (mol/mol) to the S-1 fraction during equilibrium Ca\textsuperscript{2+}-binding studies, since light chain LC\textsubscript{2} was hydrolysed during the preparation of S-1. The low Ca\textsuperscript{2+}-binding affinity value of S-1 (Fig. 4) is either the result of the Ca\textsuperscript{2+}-binding light chain not reassociating with the S-1 subfragment lacking the (S-1)–(S-2) junction or partial reassociation occurred but the Ca\textsuperscript{2+}-binding properties were no longer influenced by the heavy chains. Native myosin, conversely reassociated under these conditions (Wikman-Coffelt et al., 1979; Higuchi et al., 1978; Wikman-Coffelt, 1980; Hollosi et al., 1980) and the high Ca\textsuperscript{2+}-binding affinity value was restored. The Ca\textsuperscript{2+}-binding light chain has a different affinity for Ca\textsuperscript{2+} when complexed to the heavy chains as compared with when it is in the dissociated state (Wikman-Coffelt et al., 1979) and only binds at Ca\textsuperscript{2+} concentrations required to induce a conformational change in free light chains (Ca\textsuperscript{2+} 10\textsuperscript{-5}–10\textsuperscript{-3} M) (Morakovcic et al., 1979; Yamamoto et al., 1980).

Although myosin light chains were prepared in the presence of 8 M-urea, such procedures did not affect the Ca\textsuperscript{2+}-binding properties of the dissociated light chains as long as the denaturing agent was removed before equilibrium dialysis (Wikman-Coffelt, 1980; Wikman-Coffelt et al., 1979); however, the presence of 6 M-urea in the dialysis medium prevented all Ca\textsuperscript{2+} binding, both for native myosin (Figs. 2 and 3) and the dissociated light chains (Fig. 4).

When Ca\textsuperscript{2+} binding was analysed in the absence of the Ca\textsuperscript{2+}/EGTA buffer as described here the binding profiles for the two native myosin sites were biphasic (Figs. 2 and 3), similar to the results obtained by Watterson et al. (1979); in this latter study binding was analysed with techniques other than the addition of 45Ca to a Ca\textsuperscript{2+}/EGTA system, where labelling effects often obscure data (Wikman-Coffelt & Muhlrad, 1980). Such biphasic binding was not due to phosphorylation of myosin light chain LC\textsubscript{2}, since the purified myosin of rabbit skeletal muscle contained no phosphate after purification (Fig. 5). Conversely, although purified sheep cardiac myosin demonstrated as 30% light chain LC\textsubscript{2} phosphorylation (Fig. 6), it nevertheless gave a similar type of biphasic binding (Fig. 7). (It is unlikely that the minor contaminant protein present in cardiac myosin (Fig. 6) is a Ca\textsuperscript{2+}-binding protein, e.g. troponin or calmodulin, because it dissociates with the light chains (Fabian et al., 1977) and the dissociated light chains have a low ‘non-biphasic’ Ca\textsuperscript{2+}-binding affinity, as shown here; also the same

buffer. Conversely, H\textsuperscript{+} had no effect on the Ca\textsuperscript{2+}-binding properties of the purified light chains (Fig. 4), S-1 (Fig. 4) or SH\textsubscript{2}-N-ethylmaleimide-blocked myosin (Figs. 2 and 3).

myosin binding in 6 M-urea (△–△). The urea was first treated with Chelex-100 to remove contaminants as described in the Experimental section.

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Fig. 3. Scatchard plots of Ca\textsuperscript{2+} binding at pH 6.5 for rabbit skeletal muscle myosin
Analyses were as described in the Experimental section. For native myosin site one (●–●): $K = 0.64 \times 10^4 M^{-1}$, $n = 1.1$. Site two: $K = 2.4 \times 10^4 M^{-1}$, $n = 2.3$. Myosin treated with 2,4,6-trinitrobenzenesulphonate (○–○) gave similar values. For SH\textsubscript{2}-N-ethylmaleimide-blocked myosin (□–□) $K = 3.3 \times 10^4 M^{-1}$, $n = 2.0$. All symbols are the same as in Fig. 2. A high concentration of buffer was used in the dialysis to retain a constant pH throughout the reaction. Control and experimental samples were analysed in the same flask. Five individual experiments were carried out analysing pH differences; all analyses gave similar results. Shown also are the values for myosin binding in 6 M-urea (△–△).
degree of contamination is present after SH₂-modification (see Fig. 9); SH₂-modified myosin also has a low Ca²⁺-binding affinity. Blocking of the SH₂ thiol of the heavy chains with N-ethylmaleimide reduced the binding affinity of the oligomer to that of the dissociated light chains. There was no oligomeric dissociation with such thiol modification (Fig. 8); furthermore, pH did not affect the binding affinity of SH₂-N-ethylmaleimide-blocked myosin (Figs. 2 and 3), the dissociated light chains (Fig. 4) nor S-1 (Fig. 4). It is assumed from the ATPase activity and the gel-electrophoresis patterns (Fig. 9) that the alkali light chains were functional as LC₃ after preparation of S-1 with chymotryptic digestion; the actin-activated ATPase activity of S-1 was 0.6 µmol of P₃·min⁻¹·mg⁻¹ of myosin. Conversely, the Ca²⁺-binding properties of heavy meromyosin (Fig. 9b), where both S-1 and S-2 were present, were similar to that of native myosin (Fig. 10). Modification of the two reactive lysine residues of myosin similar to SH₁-N-ethylmaleimide blocked myosin (Srivastava et al., 1980) did not affect Ca²⁺ binding of rabbit skeletal muscle myosin (Figs. 2 and 3).

Discussion

Although the reactive SH₁ and SH₂ moieties of myosin are located on the 20 K-mol.wt. peptide of S-1 (Balin et al., 1978) and are only separated by nine amino acids (Flink et al., 1977). SH₁-N-ethylmaleimide-blocked myosin still has normal Ca²⁺-binding properties and a normal expression of isometric tension as actomyosin threads (Srivastava et al., 1980), whereas SH₂-N-ethylmaleimide-blocked myosin does not. Conversely, when the reactive lysine residue in the 27 K-mol.wt. peptide is blocked with 2,4,6-trinitrobenzenesulphonate there are normal Ca²⁺-binding properties. Such an exclusive effect of the SH₂ region of myosin heavy chains on the Ca²⁺-binding properties of the light chains is either owing to the small conformational change that occurs in this region of the molecule when it is blocked (Kameyama, 1980) or the SH₂ thiol directly participates in the heavy chain's influence on the Ca²⁺-binding properties of light chain LC₂.

The heavy meromyosin (S-1)–(S-2) junction can act as a fulcrum in generating force to push the thin filaments towards the centre of the sarcomere. Any change in molecular interaction and conformation in this region appears to affect Ca²⁺-binding, the rate of K⁺-stimulated ATPase activity and isometric tension of the synthetic actomyosin threads. This region may be important for positioning of the Ca²⁺-binding light chain so that its affinity for Ca²⁺ is increased; however, Ca²⁺ binding does not appear to be a factor involved in tension as 70% of the Ca²⁺-binding light chain can be dissociated from
myosin with no change in isometric tension (Srivastava et al., 1980). The SH$_2$ thiol is near the (S-1)–(S-2) junction (Cardinaud, 1979), perhaps in a type of tertiary folding, and blocking of this thiol causes a shift in regional charge distribution (Kameyama, 1980). This alteration in molecular interaction does not affect Ca$^{2+}$-stimulated ATPase activity in the head region nor complexing of S-1 with actin as shown in these studies; however, the alterations are sufficient to inhibit Ca$^{2+}$ binding, K$^+$-stimulated ATPase activity and isometric tension. The intact S-1 and S-2 regions are needed for influencing the Ca$^{2+}$-binding properties of light chain LC$_2$ in rabbit skeletal muscle myosin; this influence of the heavy chains on the Ca$^{2+}$-binding properties of the light chains occurs with native and heavy meromyosin, but not with S-1. With the susceptibility of the (S-1)–(S-2) junction to molecular interaction, conformational changes and/or proximity of charge distribution (Kameyama, 1980) H$^+$ also modify this region (Chiano & Harrington, 1979; Mendelson & Cheung, 1976); such modification results in a decrease in the
Ca\(^{2+}\)-binding affinity of myosin at pH 6.5 as compared with pH 7.4, as shown here. A decrease in pH may relax the positioning of light chain LC\(^{2}\). Conversely, the dissociated light chain LC\(^{2}\), like troponin (Stull & Buss, 1978), is not susceptible to changes in pH relative to Ca\(^{2+}\) binding, thus confirming that the decrease in Ca\(^{2+}\) binding of myosin with a decrease in pH is owing to the effect of H\(^{+}\) on the conformation of the heavy chains and thereby influencing its interaction with light chain LC\(^{2}\). This was further supported by the fact that the Ca\(^{2+}\) binding of SH\(^{2}\)-modified myosin, where the effect of heavy chains on Ca\(^{2+}\) binding was diminished, did not show pH-susceptibility. Since there was no pH-susceptible change in Ca\(^{2+}\) binding with S-1, but there was with heavy meromyosin, it appears that the intact (S-1)-(S-2) region is needed for influencing Ca\(^{2+}\) binding. It is not known at present how much of S-2 is needed for this influence; however, in cardiac myosin it appears that only a small peptide of the (S-1)-(S-2) junction is required (Kuwayama & Yagi, 1979).

The Ca\(^{2+}\) binding of myosin, unlike the binding of the Ca\(^{2+}\)-specific site of troponin, appears to depend on the secondary and tertiary structure of the molecule, such that binding does not take place in the presence of 6 M urea, as in the case of the Ca\(^{2+}\)-binding-specific site of troponin as was first shown by Head & Perry (1974) and later by Nagy & Gergely (1979). There are several disparities in the Ca\(^{2+}\)-binding properties of myosin as compared with troponin; the Ca\(^{2+}\)-binding loops of troponin and the corresponding site on light chain LC\(^{2}\) are similar (Kendrick-Jones & Jakes, 1977), but sufficiently different for us to believe that the overall three-dimensional structure of these regions are not the same and thus differences, as described here for
light chain LC$_2$, relative to pH and oligomer influence, would be expected. Also, the Ca$^{2+}$-binding sites on myosin are non-specific in that they can be displaced with Mg$^{2+}$ (Wikman-Coffelt, 1980; Holroyde et al., 1979), whereas there are both specific and non-specific Ca$^{2+}$-binding sites with troponin (Potter & Gergely, 1975).

Although modification of the reactive SH$_2$ thiol produces a moderate conformational change in myosin (Kameyama, 1980), which in turn alters the influence of the heavy chains on the Ca$^{2+}$-binding properties of the light chains, still other added factors such as nucleotides, which also cause a small conformational change in myosin (Seidel & Gergely, 1971; Yamamoto & Sekine, 1980; Szilagyi et al., 1979), as well as actin (Mornet et al., 1979; Yamamoto & Sekine, 1980), did not alter the Ca$^{2+}$-binding properties of myosin with analyses as described here.

Rabbit skeletal muscle myosin as an oligomer, but not as dissociated subunits, binds Ca$^{2+}$ at physiological Ca$^{2+}$ concentrations, whereas with frog both skeletal muscle myosin and the dissociated light chain LC$_2$ bind Ca$^{2+}$ at physiological Ca$^{2+}$ concentrations (Wikman-Coffelt & Srivastava, 1979). However, in all cases Mg$^{2+}$ competes for these sites at a lower but significant affinity and thus negates any type of myosin regulation (Holroyde et al., 1979; Wikman-Coffelt & Srivastava, 1979; Wikman-Coffelt, 1980). Furthermore the rate of Mg$^{2+}$ dissociation is too slow to assume a Mg$^{2+}$-Ca$^{2+}$ exchange during the contraction cycle owing to Ca$^{2+}$ transients (Bagshaw & Reed, 1977).

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References


Cardinaud, R. (1979) Biochimie 61, 807–821


Stone, D. & Perry, S. V. (1973) Biochim. J. 131, 127–137


