A synthetic peptide modeled after the calmodulin (CaM)-binding domain of rabbit skeletal muscle myosin light chain kinase, Lys-Arg-Arg-Trp-Lys Glycyl amide (M5), inhibited the CaM-independent chymotryptic fragment of the enzyme, C35 (Edelman, A. M., Takio, K., Blumenthal, D. K., Hansen, R. S., Walsh, K. A., Titanii, K., and Krebs, E. G. (1985) J. Biol. Chem. 260, 11275–11285), with a Ki of 3.2 ± 2.1 μM. Inhibition was competitive with respect to the peptide substrate Lys-Lys-Arg-Ala-Ala'-Arg-Ala- Thr-Ser-Asn'-Val-Phe-Ala and was of the noncompetitive linear mixed type with respect to ATP. M5 and homologues with a serine residue substituted at positions 9, 13, or 14 were phosphorylated with the following order of preference: M5(Ser9) > M5(Ser13) > M5(Ser14) > M5. The order of preference observed agreed with that predicted by comparison of the sequence of these peptides with the phosphorylation sites of myosin P-light chains. Both inhibition of C35 by M5 and phosphorylation of M5 and its serine-substituted homologues were severely curtailed by the addition of Ca2+ are mediated by the Ca2+-binding protein calmodulin (CaM). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
the extreme C-terminal 27 amino acids of MLCK. Since M13 that this peptide contained within it the CaM-binding region of the enzyme. Additional studies with synthetic peptides have further localized the essential determinants of high affinity CaM binding to a sequence of 17 amino acids corresponding to those occupying positions 577–593 in the enzyme (17, 35).

Comparison of the sequence of the CaM-binding domain of MLCK with the sequences of the phosphorylation sites of myosin P-light chains (28, 29) reveals significant similarities between them, especially with respect to the number and distribution of basic amino acid residues (Fig. 1, A and B). These basic residues are important determinants of the substrate specificity of MLCK (12, 26), suggesting that such a substrate-like sequence might possess the ability to bind to the active site of the enzyme. If so, it would be expected to inhibit catalysis by blocking the access of substrates, specifically myosin P-light chains. Furthermore, it could be visualized that CaM activation of MLCK would proceed via the removal of this "autoinhibitor" sequence from the active site as a result of its binding to CaM (Fig. 2). Upon dissociation of CaM in response to falling Ca2+ levels, this bifunctional autoinhibitor domain would be free once again to bind the enzyme's active site and inhibit catalysis. The effectiveness of such inhibition would be greatly augmented by the inhibitor's high local concentration, a consequence of its covalent attachment. Irreversible activation of MLCK by limited proteolysis would be explained by the removal of the autoinhibitor sequence from the active site by its cleavage from the enzyme, rather than via CaM binding.

In this study, we have utilized synthetic peptides modeled after the CaM-binding domain of MLCK to test some of the predictions of the autoinhibitor hypothesis.

**EXPERIMENTAL PROCEDURES**

**Materials—**Purchased materials included CM-Trisacryl ion-exchange resin (LKB-Produkter AB, Bromma, Sweden), phosphoric acid and high pressure liquid chromatography grade ammonium acetate (J.T. Baker Chemical Co.), PS1 ion-exchange paper (Whatman Ltd., Maidstone, England), Triton X-100 (Sigma), and Aquamax liquid scintillation mixture (Westchem, San Diego, CA). All other materials are from previously listed sources (1, 2, 7).

**Proteins and Enzymes—**The CaM-independent chymotryptic fragment of rabbit skeletal muscle MLCK, C35, was prepared as described by Edelman et al. (7). CaM was purified from bovine testes as described by Charbonneau et al. (4). Protein concentrations were determined by the method of Bradford (31), with the exception of CaM, which was determined by UV adsorption at 277 nm using an extinction coefficient of 3300 M⁻¹ cm⁻¹ (15).

**Peptides—**Synthetic peptides were prepared by the Chemical Synthesis Facility of the Howard Hughes Medical Institute at the University of Washington, Seattle. Syntheses were performed using an Applied Biosystems automated solid-phase peptide synthesizer with subsequent cleavage by hydrofluoric acid (1). Peptides were then purified by ion-exchange chromatography. Crude peptide, 50–200 μmol, was dissolved in a minimum volume of 0.2 M ammonium acetate, pH 7.0, and loaded onto a 1 × 20-cm column of CM-Trisacryl equilibrated in the same buffer. After washing with 2 column volumes of buffer, the column was eluted with a linear gradient consisting of 200 ml each of 0.2 M ammonium acetate and 1.2 M ammonium acetate, both pH 7.0. Fractions, 2–4 ml each, were collected and peptides detected by their UV absorption at 280 nm. Peak fractions were pooled and their amino acid composition determined. Peptides with the correct composition were then sequenced both to confirm their identity and to check for impurities, which appeared in the form of underlying sequences since capping was not employed in the sequence protocol. By this criteria all peptides used were at least 90% pure. For M5 and 2α homologues, the most common contaminant was a peptide lacking one of the two arginine residues located at positions 2 and 3. Amino acid analysis and peptide sequence analysis were performed as described by Takio et al. (34). Prior to use, peptides were lyophilized to remove the ammonium acetate and dissolved in 50 mM MOPS, pH 7.0. Peptide concentrations were determined by amino acid analysis. In the case of M5 and related peptides, this was sometimes checked by measuring UV absorbance at 280 nm using an extinction coefficient of 5550 M⁻¹ cm⁻¹ (1).

**Assay of MLCK Activity—**Activity was determined by measuring the time-dependent incorporation of [32P]phosphate from [γ-32P]ATP into the substrate peptide Lys-Lys-Arg-Ala-Ala-Arg-Ala-Thr-Ser-Aux-Val-Phe-Ala (MLC peptide), by a modification of the procedure of Kemp and Pearson (12). MLC peptide represents a consensus sequence of the important specificity determinants found in the phosphorylation sites of myosin P-light chains and as such is an

![Diagram](https://example.com/diagram.png)

**FIG. 2. The autoinhibitor hypothesis for the CaM-mediated activation of skeletal MLCK.** The hatched circles correspond to the amino-terminal rodlike tail domain of MLCK. The open circles represent the catalytic domain, and the darkened circles represent the CaM-binding/autoinhibitor domain. The large circle with CaM in the center represents calmodulin.
excellent substrate for MLCK from smooth and skeletal muscle (12).
Assays were performed at 25°C in a volume of 32 μl containing 50
mM MOPS (pH 7.0), 12.5 mM MgCl₂, 1 mM dithiothreitol, 2 mM
EGTA, 1 mg/ml bovine serum albumin, 1% (v/v) Triton X-100, 0.2
μg/ml C35, and varying amounts of MLC peptide and [γ-32P]ATP
(sp. act. 4000 cpm/μmol), as well as other compounds as noted. The concentrations of MLC peptide and ATP are
listed in the individual table and figure legends. To ensure that the incorporation of phosphate into MLC peptide was linear with time, aliquots (8 μl) were removed at three separate times from each assay.

These were spotted onto 2 × 2-cm squares of P81 ion-exchange chromato-graphic paper and immediately immersed in 150 mM H₃PO₄
to quench the reaction. Unreacted ATP, inorganic phosphate, etc.
were removed from the peptide, which adheres to the paper, by
washing for periods of 30 min to 12 h in 5–8 changes (400 ml each)
of 150 mM H₃PO₄ (30). The paper squares were suspended in a wire
basket in order to allow the wash mixture to be stirred without damage to the paper. The paper squares were then washed for 1 min in 95% ethanol, air-dried, and counted in 4 ml of scintillation mixture
in 95% ethanol, air-dried, and counted in 4 ml of scintillation mixture.

RESULTS

The MLCK CaM-binding domain peptide, M5, was tested for its inhibitory properties. M5 has the following sequence: Lys-Arg-Arg-Tyr-Lys'-Lys'-Asp-Phe-Ile-Ala'-Val-Ser-Ala-Ala-Aan³⁵, Arg-Phe-Gly-NH₂. The first 17 amino acids of this peptide are identical to those found in positions 577–593 of rabbit skeletal muscle MLCK (35). The glycyl amide group was added in order to eliminate the negative charge of the terminal carboxyl group, as no such charge is present at this position in the native protein. M5 binds CaM with the same high affinity (Kᵣ ≈ 1 nM) as does the somewhat longer peptide M13 (27) and MLCK itself (27).

The inhibitory properties of M5 were examined using the CaM-independent chymotryptic fragment of MLCK, C35. This form of the enzyme was used, rather than native MLCK, for two reasons. First, we wished to examine the ability of M5 to inhibit catalysis by MLCK independent of the peptide’s ability to inhibit CaM activation of the enzyme (2). Second, we wished to compare the inhibitory properties of both the free and the CaM-bound forms of M5. Since these experiments required a CaM-independent source of MLCK activity, C35 was selected. The C35 preparations possess a disadvantage in having a specific activity only about 5% that of native MLCK (7). However, the Kᵣ values of the chymotryptic fragment and the intact enzyme for rabbit skeletal muscle myosin light chains and ATP were almost the same (11.9 ± 0.9 μM (n = 5) and 284 ± 59 μM (n = 7) for C35 versus 10–20 μM for MLCK (27), respectively). Therefore, we felt that C35 represented a suitable catalytic analogue of MLCK.

Initial experiments revealed that inhibition of C35 was detectable using micromolar concentrations of M5. Double reciprocal analysis showed that this inhibition was competitive with respect to MLC peptide (Fig. 3), but noncompetitive linear mixed type (32) with respect to ATP (Fig. 4). Secondary plots of the data also indicated that the inhibition was competitive in nature. Dixon plots displayed evidence of intersection in the fourth quadrant (Fig. 5, top), and replots of the slope of these plots as a function the 1/MLC peptide were linear and intersected the origin (Fig. 5, bottom) as predicted for competitive inhibition. Other secondary plots graphing the slope or apparent Kᵣ generated from the double reciprocal plot as a function of M5 concentration were linear (data not shown). These various replots all suggest that the inhibition was competitive and results from the formation of a simple 1:1 complex possessing no residual catalytic activity. The Kᵣ determined from the average of three experiments of the type represented in Fig. 3 and 5 was 3.2 ± 2.1 μM.

The effect of CaM binding on the ability of M5 to inhibit C35 is shown in Table I. Under conditions where 65–69% of C35 activity was inhibited by M5, addition of a 5-fold excess of CaM over peptide resulted in near complete abolition of the latter’s inhibitory potency. This effect was, however, found to be independent of Ca²⁺, suggesting that while Ca²⁺ is needed for the high affinity binding of M5 and related peptides (2), CaM retains a finite (estimated Kᵣ ≈ 10⁻⁸ M) affinity for them in the absence of Ca²⁺.

If M5 inhibited C35 by binding to the enzyme’s myosin P-light chain substrate binding site, we reasoned that an appro-

FIG. 3. Inhibition of C35 by M5: MLC peptide as variable substrate. Activity was assayed as described under “Experimental Procedures” in the presence of varying concentrations of both MLC peptide and M5. The concentration of ATP was held constant at 1.0 mM. The concentrations of MLC peptide employed were 20, 25, 40, 80, or 160 μM. The concentrations of M5 used were 0 μM (X), 4 μM (■), 8 μM (▲), or 12 μM (□).

FIG. 4. Inhibition of C35 by M5: ATP as variable substrate. Activity was assayed as described under “Experimental Procedures” in the presence of varying concentrations of both ATP and M5. The concentration of MLC peptide was held constant at 240 μM. The concentrations of ATP employed were 100, 125, 167, 250, or 500 μM. The concentrations of M5 used were 0 μM (X), 8 μM (■), 16 μM (▲), or 24 μM (□).
Autoinhibition of Myosin Light Chain Kinase

The activity for each set of additions of Caz+, EGTA, or CaM in the presence of M5 peptide to that in its absence. The presence or absence of each is indicated above the data from Fig. 3 representing the following concentrations of M5 peptide: 20 μM (x), 25 μM (Δ), 40 μM (Δ), 80 μM (■), and 160 μM (□). Bottom, a secondary plot of the Dixon plot displaying the slope of the Dixon plot as a function of 1/MLC peptide concentration.

Table 1: Effect of calmodulin on the inhibition of C35 by M5 peptide

<table>
<thead>
<tr>
<th>Additions</th>
<th>Ca²⁺</th>
<th>EGTA</th>
<th>CaM</th>
<th>M5</th>
<th>Relative activity (%)</th>
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<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 ± 3</td>
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<td>-</td>
<td>+</td>
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<td>-</td>
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<td>31 ± 2</td>
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<td>-</td>
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<td>-</td>
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<td>100 ± 3</td>
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<td>-</td>
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<td>35 ± 2</td>
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<td>-</td>
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<td>100 ± 3</td>
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<td>+</td>
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<td>92 ± 8</td>
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</tbody>
</table>

* Assays were performed as described under “Experimental Procedures” with the following modifications. Instead of 2 mM EGTA, one or more of the following were present at the concentrations given in parentheses: CaCl₂ (0.5 mM), EGTA (2 mM), CaM (35 μM), and/or or M5 (20 μM). The presence or absence of each is indicated above by use of a + or −, respectively. The concentrations of MLC peptide and ATP were 40 μM and 1.0 mM, respectively.

In all values represent the mean of three determinations plus or minus standard error. The relative activity is defined as the ratio of the activity for each set of additions of Ca²⁺, EGTA, or CaM in the presence of M5 peptide to that in its absence.

100% relative activity corresponds to an actual rate of 0.89 pmol/min.

The incorporation of 32P into M5 and variants thereof was measured under essentially the same conditions described under “Experimental Procedures” for the assay of MLCK activity. Modifications included raising the total volume to 48 μl, increasing the C35 concentration to 21 μg/ml, and substituting the peptides listed below for MLC peptide. The concentrations of peptides and ATP were 50 μM and 1.0 mM, respectively. The peptide variants employed included M5, which has a serine at position 12 (M5, x), as well as homologues with a serine substituted for the alanine present at either position 13 (M5(Ser³), △) or 14 (M5(Ser⁴), ■) or for the isoleucine at position 9 (M5(Ser⁹), □).

Fig. 5. Secondary plots of inhibition of C35 by M5: MLC peptide as variable substrate. Top, the data from Fig. 3 represented as a Dixon plot giving 1/velocity as a function of M5 concentration for the following concentrations of MLC peptide: 20 μM (x), 25 μM (Δ), 40 μM (Δ), 80 μM (■), and 160 μM (□). Bottom, a secondary plot of the Dixon plot displaying the slope of the Dixon plot as a function of 1/MLC peptide concentration.

Fig. 6. Phosphorylation of M5 and serine-substituted homologues by C35. The incorporation of 32P into M5 and variants thereof was measured under essentially the same conditions described under “Experimental Procedures” for the assay of MLCK activity. Modifications included raising the total volume to 48 μl, increasing the C35 concentration to 21 μg/ml, and substituting the peptides listed below for MLC peptide. The concentrations of peptides and ATP were 50 μM and 1.0 mM, respectively. The peptide variants employed included M5, which has a serine at position 12 (M5, x), as well as homologues with a serine substituted for the alanine present at either position 13 (M5(Ser³), △) or 14 (M5(Ser⁴), ■) or for the isoleucine at position 9 (M5(Ser⁹), □).

Fig. 7. Effect of CaM on the phosphorylation of a serine-substituted homologue of M5. The measurement of [32P]phosphate incorporation into peptide M5(Ser⁹) was performed as described in the legend of Fig. 6, except that the assay mixtures contained either 0.5 mM CaCl₂ (−CaM, ■) or 0.5 mM CaCl₂ plus a slight stoichiometric excess, 53 μM, of CaM (+CaM, □). At the indicated times aliquots, 6 μl of each, were removed and 32P incorporation into peptide determined as described for MLC peptide under “Experimental Procedures.”

Privately constructed homologue should be phosphorylated by the enzyme in the presence of ATP. Comparison of the amino acid sequence of M5 with those of the phosphorylation sites of myosin P-light chains and synthetic peptide substrates indicated two potential alignments of “maximal homology” (Fig. 1). In the first, based upon optimizing similarity with myosin P-light chain from skeletal muscle sources (29), the phosphorylated serine in the light chain was positioned opposite Ala¹³ in M5, or in a less likely alignment, Ala¹⁴ (Fig. 1A). The second, based on smooth muscle myosin light chains (28) and MLC peptide (12), placed the phosphorylated serine opposite Ile⁶ (Fig. 1, B and C). Since skeletal muscle MLCK readily phosphorylates myosin P-light chains from both skeletal and smooth muscle sources, as well as MLC peptide, in vitro (33), homologues of M5 containing serine at position 9, 13, or 14 were synthesized. These were tested, along with M5 itself (which already has a serine at position 12), as substrates for C35. As can be seen in Fig. 6, all four peptides could be phosphorylated, with the apparent order of preference M5(Ser³) > M5(Ser⁴) > M5(Ser⁹) > M5. The specific activ-
ity of C35 toward these peptides was quite low, nearly 1000-fold lower than toward myosin P-light chains or MLC peptide. The $K_i$ of C35 for the most readily phosphorylated peptides, M5(Ser)$^9$ and M5(Ser)$^{13}$, was similar to those of good peptide and protein substrates, however, approximately 15–20 μM for each (data not shown).

The effect of CaM binding upon the properties of M5 and its homologues as substrates for C35 was also examined. As can be seen in Fig. 7, the addition of a small stoichiometric excess of CaM over peptide M5(Ser)$^9$ dramatically decreased its rate of phosphorylation. Similar decreases in rate were also observed using the other three peptides as substrate (data not shown).

**Discussion**

M5, a synthetic peptide modeled after the CaM-binding domain of rabbit skeletal muscle MLCK, inhibited the CaM-independent chymotryptic fragment of the enzyme, C35, with a $K_i$ of 3.2 ± 2.1 μM. Based on the following criteria, this inhibition appears to result from the binding of M5 to the enzyme’s myosin P-light chain substrate binding site. First, inhibition was competitive with respect to MLC peptide, but was of the noncompetitive linear mixed type with respect to ATP. This pattern of inhibition matches that predicted from the kinetic mechanism of MLCK for an active site-directed inhibitor (10). Second, the $K_m$ for M5 peptide was similar in magnitude, and in fact lower than, the $K_m$ of C35 for skeletal muscle myosin P-light chains (12 μM). Third, secondary plots describing the inhibition suggest that the inhibition was competitive and resulted from the formation of a 1:1 M5-enzyme complex which possessed no residual catalytic activity. Fourth, M5 and homologues synthesized with serine substituted for either Ile$^9$, Ala$^{13}$, or Ala$^{14}$ (M5(Ser)$^9$, M5(Ser)$^{13}$, and M5(Ser)$^{14}$, respectively) were phosphorylated by C35 with the order of preference predicted by comparison of their sequences to those of peptide and protein substrates. The $K_i$ values estimated for the two most readily phosphorylated peptides, M5(Ser)$^9$ and M5(Ser)$^{13}$, were comparable to those for myosin P-light chains and similar in magnitude to the $K_i$ of M5. This suggests that M5 and its homologues were able to bind C35 in a substrate-like manner and do so at concentrations similar to those at which M5 was inhibitory.

It should be noted that the specific activity of C35 toward M5 and its homologues was nearly 1000-fold lower than its specific activity toward myosin P-light chains or MLC peptide. This is not entirely unexpected, since the properties which render a molecule an effective active site-directed inhibitor do not necessarily coincide with those which render it a good substrate. For example, a serine-substituted peptide modeled after the heat-stable inhibitor of cAMP-dependent protein kinase turned out to be a very poor substrate of the enzyme (31). Conversely, attempts to develop inhibitors of this same kinase based upon the sequence of the excellent peptide substrate Kempetide (Leu-Arg-Arg-Ala-Ser-Leu-Gly) met with little success (8, 11).

The ability of CaM to block both the inhibition of C35 by M5 and the phosphorylation of M5 and related homologues indicates that these peptides have little or no binding affinity for the enzyme’s active site following their complexation by CaM. This is significant, since it provides a mechanism by which CaM could relieve autoinhibition and activate MLCK. The abolition of M5-enzyme interaction upon complexation of the peptide to CaM was presumably the result of the spatial rearrangement of important binding determinants in M5 as well as their physical masking. The former would occur upon the transition of the peptide from an extended conformation lacking ordered secondary structure to an α-helical one upon binding CaM (17, 18). CaM binding produces a similar increase in the α-helical content of MLCK and its CaM-dependent “head” fragments (25), suggesting that the enzyme’s CaM-binding domain (upon which M5 was modeled) may undergo a similar conformation change.

The question arises as to whether the bifunctional nature of M5 represents a property unique to it and its homologues or was merely a consequence of similarities between the primary sequence requirements for CaM and MLCK substrate binding. Four lines of evidence suggest that M5 is uniquely adapted for its dual role. First, another potent CaM binding peptide, mastoparan (21), was an extremely poor inhibitor of C35. Second, the numerous studies performed on peptide substrates and competitive inhibitors of the myosin light chain kinases, such as MLC peptide, have failed to reveal any high affinity CaM antagonist ability endogenous to them (12, 26). Third, partial proteolytic digestion of MLCK with chymotrypsin produced an intermediate form between the CaM-dependent C37 and CaM-independent C35. This form, C36, was still fully CaM-dependent but required much higher concentrations of CaM to become activated (7). This suggests that residues essential for the high affinity binding of CaM were removed without affecting those essential for inhibition of activity. Fourth, although both CaM binding and MLCK substrate binding require clusters of positively charged amino acids as their most obvious common structural determinant, the spatial requirements for the arrangement of these clusters probably differ greatly. Substrates and inhibitors of MLCK require a fairly strict spacing of basic residues within their linear sequence (12, 26), presumably reflective of the adoption of an extended conformation when bound to the enzyme active site. CaM binding peptides require that their basic residues be distributed such that they all reside on one face of an amphipathic helix, the conformation which these peptides adopt upon binding to CaM (6). This three-dimensional arrangement can be accommodated by a large number of distinct spacings within a peptide’s linear sequence, only some of which conform to those compatible with the specificity determinants of the MLCK active site.

To summarize, the above results indicate that M5, a synthetic peptide modeled after the CaM-binding domain of rabbit skeletal muscle MLCK, is a CaM-regulated competitive inhibitor of the active chymotryptic fragment of the enzyme, C35. Such behavior is consistent with the concept that the CaM-binding domain of native MLCK functions as a CaM-regulated autoinhibitor of enzymatic activity (Fig. 2) and suggests that the functions of CaM binding and enzyme inhibition, therefore, reside in identical or highly overlapping, rather than discrete, domains as had previously been speculated (7). However, although consistent with this mechanism, these results do not rule out alternative mechanisms such as a CaM-induced change in the structure of the MLCK active site itself or the CaM-induced movement of portions of the protein which might sterically block the active site. The final word on the relevance of the inhibitory properties of M5 to the mechanism of the CaM-regulated activation/inactivation of skeletal muscle MLCK must, therefore, await further investigation by x-ray crystallography and other means. However, several things are worthy of note. The first is that the autoinhibitor hypothesis requires that the CaM-binding domain of MLCK must be suitably positioned and possess sufficient configurational flexibility to swing into and out of the enzyme’s active site. The CaM-binding domain of MLCK is located at the extreme C terminus of the enzyme, a region

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* J. P. Kennelly and K. Alexander, unpublished observations.
more likely to possess a high degree of flexibility than areas nearer the center of the polypeptide chain. Furthermore, circular dichroism and nuclear magnetic resonance data suggest that M5 and related peptides adopt a flexible extended conformation in solution (17, 18) and thus might be expected to do so in MLCK. Second, the proposed model is consistent with the observation that skeletal muscle MLCK possesses a catalytic core which expresses CaM-independent activity following the proteolytic removal of the enzyme’s CaM-binding domain (7). In this circumstance, activation would be attributable to the removal of the autoinhibitor from the active site by physical cleavage, rather than CaM binding. Partial proteolysis of other CaM-regulated enzymes such as CaM-dependent phosphodiesterase (19) and calcineurin (24) also results in the appearance of CaM-independent active forms of these enzymes. Thus, it is possible that other CaM-modulated enzymes may possess a similar domain organization as skeletal muscle MLCK and may, therefore, be activated in a manner similar to that postulated here.

Perhaps the strongest support for the autoinhibitor hypothesis appeared while this manuscript was in the late stages of preparation in a report detailing the CaM and substrate antagonist abilities of synthetic peptides toward the smooth muscle isozyme of MLCK (13). These synthetic peptides were modeled after portions of the putative CaM-binding domain of smooth muscle MLCK or, in one instance, a portion of the CaM-binding domain of the skeletal muscle isoform (residues 577–587 as opposed to residues 573–593 for M5). Many of these peptides were found to be potent inhibitors of the CaM activation of smooth muscle MLCK as well as competitive inhibitors of the activated enzyme with respect to myosin P-light chains. Although the ability of the smooth muscle MLCK peptides to inhibit the CaM activation of the enzyme could be overcome by the addition of excess CaM, their active site-directed inhibitory capabilities were not. Kemp et al. (13) postulated on the basis of these observations that the CaM-binding domain of smooth muscle MLCK was a bifunctional domain which bound to the active site in the absence of CaM as a “pseudosubstrate” and blocked enzymatic activity. Thus, synthetic peptides modeled after the CaM-binding domains of smooth and skeletal muscle MLCK exhibit similar inhibitory properties toward their respective parent enzymes, and this has led to the postulation of similar autoinhibitor-type mechanisms for each. These parallels take on added significance when one considers that the skeletal and smooth muscle isoforms of MLCK differ greatly enough in molecular weight, antigenic properties, and molecular architecture to be considered, in many ways, distinct CaM-regulated proteins (33).

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REFERENCES