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Interaction of Calmodulin and a Calmodulin-Binding Peptide from Myosin Light Chain Kinase: Major Spectral Changes in Both Occur as the Result of Complex Formation†

Rachel E. Klevit,*,‡ Donald K. Blumenthal,§ David E. Wemmer,‡ and Edwin G. Krebs§
Department of Chemistry and Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195
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ABSTRACT: Many different enzymes are activated by direct interaction with calmodulin; this interaction is thought to occur through a distinct calmodulin-binding domain in each of these enzymes. We have recently reported the sequence of a 27-residue peptide (denoted M13), derived from skeletal muscle myosin light chain kinase (MLCK), that exhibits the properties expected of a calmodulin-binding domain [Blumenthal, D. K., Takio, K., Edelman, A. M., Charbonneau, H., Titani, K., Walsh, K. A., & Krebs, E. G. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3187–3191]. The interaction between chemically synthesized M13 peptide and calmodulin has been studied by circular dichroism (CD) and proton nuclear magnetic resonance (NMR) spectroscopy. In the presence of Ca^{2+}, the observed ellipticity of an equimolar mixture of M13 and calmodulin is much greater than the sum of the ellipticities of the two isolated proteins. In the absence of Ca^{2+}, the measured ellipticity of the mixture is approximately the sum of the two components. Addition of the peptide to calmodulin causes dramatic changes in the proton NMR spectrum; at a 1:1 molar ratio, no evidence of either free peptide or free calmodulin is observed. Moreover, these data demonstrate that a unique species of the M13–calmodulin complex is formed, indicating that the peptide binds to calmodulin in only one way. The many resonances affected by M13 binding include residues in both halves of the calmodulin molecule. The observed CD and NMR effects suggest that secondary and tertiary conformational changes occur both in M13 and in calmodulin upon complex formation. Thus, changes in calmodulin tertiary structure following protein binding may represent an additional step in the presently accepted mechanism for calmodulin-dependent activation of MLCK and other target proteins.

The Ca^{2+}-binding protein calmodulin regulates a wide variety of enzymes and processes [for reviews, see Manalan & Klee (1984) and Klee & Vanaman (1982)]. Although the general scheme for the mechanism of regulation of many enzymes by calmodulin (eq 1) has been accepted for some time, little is known at the structural level about the interactions that occur between calmodulin and its various target proteins.

$$CaM + 4Ca^{2+} \rightleftarrows Ca^{2+}_4\cdot CaM + enzyme_{inactive} \rightleftarrows Ca^{2+}_4\cdot CaM\cdot enzyme_{active} \quad (1)$$

Recently we reported the sequence of a 27-residue peptide derived from skeletal muscle myosin light chain kinase (MLCK)[1] that shows the properties expected of a calmodulin-binding domain (Blumenthal et al., 1985). The studies of Edelman et al. (1985) involving limited proteolysis of the intact enzyme also point to this region in MLCK as being involved in calmodulin binding. The peptide, called M13, is isolated

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‡ Department of Chemistry.
§ Howard Hughes Medical Institute.

[1] Abbreviations: Aoc, *tert*-amyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; CaM, calmodulin; CD, circular dichroism; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; HPLC, high-performance liquid chromatography; MLCK, myosin light chain kinase; MOPS, 4-morpholinepropanesulfonic acid; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

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from the carboxyl terminus of MLCK (Takio et al., 1985) and binds to calmodulin with high affinity ($K_D \sim 1$ nM). The sequence has a high percentage of basic and hydrophobic residues and is predicted to form an amphipathic helix (Blumenthal et al., 1985).

M13 lacking the C-terminal methionine has been synthesized in order to study its interaction with calmodulin:

K^1-R-R-W-K-K-N-F-I-A^{10}-V-S-
A-A-N-R-F-K-K-I^{20}-S-S-S-G-A-L

The synthetic peptide inhibits calmodulin activation of MLCK with the same affinity as M13 derived from MLCK by CNBr cleavage. Experiments utilizing intrinsic tryptophan fluorescence and calmodulin-affinity chromatography established the 1:1 stoichiometry and Ca^{2+} dependency of the interaction with calmodulin (Blumenthal et al., 1985). We describe here studies utilizing high-resolution proton NMR[1] and circular dichroism (CD) to explore the interaction between synthetic M13 and calmodulin. The results of these studies indicate that secondary and tertiary structural changes occur as the result of complex formation between calmodulin and a peptide derived from a physiologically relevant target protein.

Materials and Methods

M13 was synthesized by standard solid-phase methods (Glass, 1983) using a Beckman 990B automated peptide synthesizer. Boc-L-leucine coupled to 1% cross-linked polystyrene (0.51 mequiv/g) was purchased from Vega Biochemicals. The synthesis was performed at 0.5-mequiv scale with a 2.5-fold molar excess of each amino acid derivative during coupling. The following side-chain-protected amino acids were used: α-Boc-L-serine (benzyl), α-Boc-L-lysine [ε-(2-chlorobenzyloxycarbonyl)], α-Boc-L-asparagine (xanthenyl), and α-Aoc-L-arginine (4-toluenesulfonyl). The other Boc-amino acid derivatives used had no side-chain protection. α-Boc-L-tryptophan was used without protection, but 2% ethanedithiol was added to the Boc deprotection reagent (30% trifluoroacetic acid/CH_2Cl_2) following its incorporation into the peptide to minimize oxidation. All residues were double-coupled by using equimolar amounts of dicyclohexylcarbodiimide and Boc-amino acid in 15 mL of CH_2Cl_2, except for asparagine, which was triple-coupled in 40% dimethylformamide/CH_2Cl_2. The synthesis protocol that was followed is described in more detail by Glass (1983) with the exception that 30% trifluoroacetic acid/CH_2Cl_2 was used for deprotection. The yield of peptide estimated by weighing the dry peptide–resin was 38%, assuming that all mass gain was into a single peptide. Cleavage and side-chain deprotection were effected by using anhydrous HF containing 20% anisole for 45 min at 0 °C. Purification involved ion-exchange chromatography with SP-Trisacryl (LKB), gel filtration on Sephadex G-10 (Pharmacia), and reversed-phase HPLC (Vydac C-4). Peptide purity was estimated to be at least 98% by reversed-phase HPLC (Vydac C-4, 214TP54 analytical column; gradient at 0.22%/min from 0.1% trifluoroacetic acid/H_2O to 0.1% trifluoroacetic acid/acetonitrile at 1 mL/min) as monitored by absorbance at 206 nm and fluorescence (excitation = 280 nm, emission > 300 nm). Edman degradation also indicated >95% purity (i.e., no preview sequences were observed). The composition and sequence of the peptide were verified by amino acid analysis, Edman degradation, and NMR spectroscopy. Calmodulin was prepared from bovine testis by a described method (Klevit & Vanaman, 1984).

The concentration of M13 was determined spectrophotometrically by using $A_{280nm} = 5550\ M^{-1}\ cm^{-1}$ for the single tryptophanyl residue in the peptide; quantitation by this technique was found to be in good agreement with values determined by amino acid analysis. The concentration of calmodulin was determined by amino acid analysis. The circular dichroic spectra were obtained on a modified Cary 60 spectropolarimeter. The concentrations of calmodulin and M13 for these studies were both 75 μM. The spectra were obtained at ambient temperature in 20 mM MOPS, pH 7.0, and 100 mM NaCl, with a path length of 1 mm. Scans were taken from 310 to 200 nm in 0.2-nm steps, with a 1.5-nm bandwidth. The signal at each step was automatically sampled and signal-averaged. Sampling time at each wavelength was adjusted to maintain a constant signal-to-noise ratio across the spectrum. In the spectra shown, the data points at 0.5-nm steps were connected by hand after computer application of a four-point smoothing routine.

Table I: Circular Dichroism of Calmodulin, M13, and Calmodulin–M13

sample	Ca^{2+} [a]	θ_{222nm} (deg)	$[\theta]_{222nm}$ (deg cm^2 dmol^{-1})	% helix[b]
calmodulin	+	−0.164	−14 800	41
	−	−0.128	−11 500	31
M13	+	−0.003	−1540	(0)
calmodulin + M13	+	−0.231	−17 700[c]/32 820[d]	51[c]/101[d]
	−	−0.155	−11 900[c]/12 300[d]	32[c]/33[d]

[a] Measurements were made with 1 mM Ca^{2+} (+) or 10 mM EDTA added (−). [b] Percent helix calculated according to Chen & Yang (1971) by using the expression $\text{fraction}_{helix} = -([\theta]_{222} + 2340)/30300$. [c] Calculated by assuming ellipticity change in both peptide and calmodulin structures. [d] Calculated by assuming ellipticity change due to change in peptide structure only.

For the NMR titrations, the concentrations of M13 and calmodulin were matched, and equal volumes were lyophilized separately. The NMR sample was dissolved in 500 μL of 1 mM Tris and 5 mM $CaCl_2$, pH 6.5 (uncorrected for isotope effect), in 99.96% isotopic purity D_2O, giving a final concentration of 0.5 mM. The titrant was dissolved in 50 μL of the same buffer in D_2O, so that 5-μL aliquots corresponded to 0.1 molar equiv of the NMR sample. NMR spectra (500 MHz) were obtained on a Bruker WM-500 spectrometer at 27 °C. The number of transients that were signal-averaged depended on the nature of the sample and experiment but was usually between 400 and 600. A 90° observation pulse was used, and the residual solvent resonance was presaturated for 1 s during the recycle delay. All spectra were processed identically, without line broadening or resolution enhancement. Spectra within a titration experiment were normalized to the first spectrum of the set prior to Fourier transformation. No correction was made for the small dilution error. Such normalized spectra could then be used to generate difference spectra, with the fraction of spectral intensity subtracted equal to the mole fraction of titrant present in the spectrum.

Results

Circular Dichroism. Figure 1 and Table I show the results of circular dichroic measurements made on calmodulin alone, M13 alone, and the two together. The mean residue ellipticity at 222 nm measured for calmodulin in the presence and absence of Ca^{2+} agrees well with published values [reviewed in Klee & Vanaman (1982)]. The mean residue ellipticity for M13 alone is very low, and the CD spectrum of the peptide indicates little, if any, periodic secondary structure. Due to sample constraints, the CD spectrum of M13 in the absence of Ca^{2+} was not obtained. NMR spectra of the isolated peptide in the presence and absence of Ca^{2+}, however, were identical, indicating that the structure of isolated M13 is the same in the presence and absence of Ca^{2+} (data not shown). The

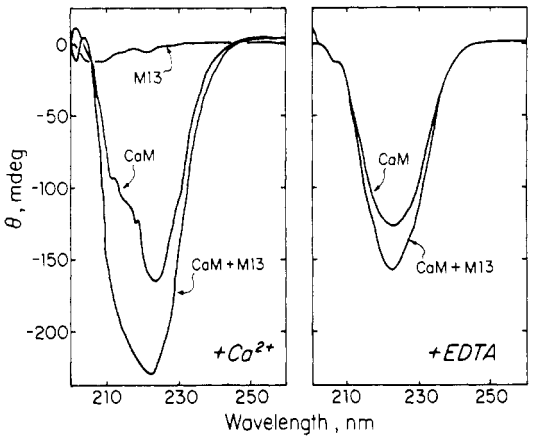

FIGURE 1: Circular dichroism spectra of calmodulin and M13. CD spectra were obtained for calmodulin, M13, and an equimolar mixture of calmodulin and M13, as described under Materials and Methods. The spectra in the left panel were obtained in the presence of 1 mM Ca^{2+}; the spectra in the right panel were obtained after addition of 10 mM EDTA to the same samples.

measured ellipticity at 222 nm for an equimolar mixture of M13 and calmodulin in the presence of Ca^{2+} is much greater than the sum of the individual ellipticities, indicating a significant increase in α-helix upon complex formation. In the presence of EDTA, however, the ellipticity of the mixture is only slightly greater than the sum of the component ellipticities. Thus, the formation of a complex with high α-helical content is Ca^{2+}-dependent.

There is no way to determine from CD measurements alone how much of the increase in ellipticity can be attributed to M13 and how much to calmodulin. The calculated helical content of the M13–calmodulin complex averaged over all residues is 51% (Table I). However, if the assumption is made that all of the change in observed ellipticity is due to the peptide, then the mean residue ellipticity for M13 in the complex is $-32\,820$ deg cm^2 dmol^{-1}, a value that would be expected if the peptide assumed an α-helical configuration over its entire length. If it is assumed that all of the observed change is due to calmodulin, the mean residue ellipticity is $-21\,000$ deg cm^2 dmol^{-1}, which corresponds to a 20% increase in the α-helicity of calmodulin. The last of these interpretations (i.e., that the increase is due solely to calmodulin) is unlikely on the basis of the NMR results presented below.

NMR Spectroscopy. Figure 2 shows the results of adding calmodulin to M13 in the presence of Ca^{2+}. The spectrum of M13 alone (bottom spectrum) consists of sharp, well-defined proton peaks occurring at spectral positions expected of the free constituent amino acids, in accord with the CD results showing that the isolated peptide in solution is devoid of regular structure. The addition of calmodulin causes new resonances to appear upfield of the aromatic M13 resonances. The new peaks are significantly broader than those of M13 and occur at positions where isolated calmodulin has no peaks, indicating that these resonances are from protons in a peptide–calmodulin complex. The resonances of free M13 do not shift or broaden during the titration, but simply decrease in intensity, indicating that M13 has a slow off-rate from the complex. This slow exchange behavior is consistent with the high-affinity binding constant ($K_D \sim 1$ nM) previously reported (Blumenthal et al., 1985). The middle two spectra of Figure 2 confirm that the stoichiometry of binding of M13 to calmodulin is 1:1. The spectrum labeled [M13]:[CaM] = 1.0:0.5 is equivalent to [M13]:[CaM] = 2:1. At this ratio, the resonances due to free M13 are clearly observed, indicating that the molar excess of M13 does not associate with the 1:1 complex. The spectrum

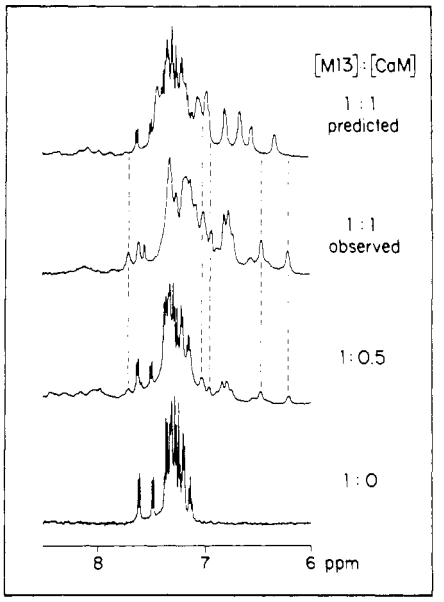

FIGURE 2: Comparison of observed vs. predicted spectra of the calmodulin–M13 complex. Shown is the aromatic region of the 500-MHz NMR spectrum of M13 with successive additions of calmodulin in the presence of 1 mM Ca^{2+}. The samples for the titration were prepared as described under Materials and Methods. The top spectrum (predicted) was generated by addition of the spectra of isolated M13 and calmodulin. Dotted lines follow the appearance of new peaks due to the M13–calmodulin complex.

labeled [M13]:[CaM] = 1:1 (observed) does not contain any peaks due to free M13. This is consistent with both a 1:1 stoichiometry and a high-affinity interaction. The top spectrum in Figure 2 represents that which is predicted if the two molecules do not interact. The experimentally observed spectrum for the 1:1 complex is clearly different from the predicted spectrum. Differences are especially marked in the upfield-shifted aromatic resonances of calmodulin (see below) and the two downfield doublets due to the tryptophanyl protons of M13.

Figure 3 shows the results of adding increasing amounts of M13 to a solution of calmodulin in the presence of Ca^{2+}. A major spectral rearrangement occurs upon complex formation. The bottom spectrum is that of isolated calmodulin and contains previously published resonance assignments for the upfield-shifted peaks (Dalgarno et al., 1984b). The ring resonances of Tyr-138 slowly disappear, while new peaks appear slightly upfield. These new peaks are likely to be due to Tyr-138 in the complex as they exhibit NOEs to each other (data not shown). All of the upfield-shifted peaks, containing resonances of ring protons from Phe-89, Phe-16, Tyr-99, Phe-68, Phe-141, and Phe-65, are affected by M13 binding as are a variety of unassigned phenylalanine resonances. The peaks in the upfield portion of the spectra ($\sim$5.2 ppm) are due to C^{α} protons of the Ca^{2+}-binding loops of calmodulin (Dalgarno et al., 1984b). The furthest downfield of those peaks, which has been shown to be derived from one of the two N-terminal Ca^{2+}-binding loops, gradually disappears during the titration. A new peak with the intensity of two protons forms at the downfield edge of this region. A large number of aliphatic peaks, including several methionyl methyls and trimethyllysine-115, are also affected by M13 (data not shown). The linear relationship between the amount of M13 added (up to a 1:1 molar ratio) and both the appearance of resonances attributable to the complex and the disappearance of resonances from free calmodulin indicates the formation of a *single species* of high-affinity complex composed of equimolar amounts of M13 and calmodulin.

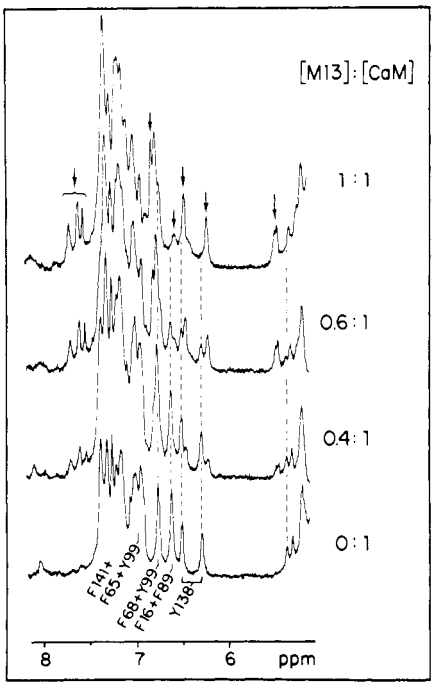

FIGURE 3: Titration of calmodulin with M13. Shown are the aromatic resonances and the downfield-shifted C^{α}-proton resonances of Ca^{2+}-saturated calmodulin following successive additions of M13, as described under Materials and Methods. The arrows point to some of the new peaks that appear during the titration. The dotted lines indicate peaks in free calmodulin that disappear during the titration.

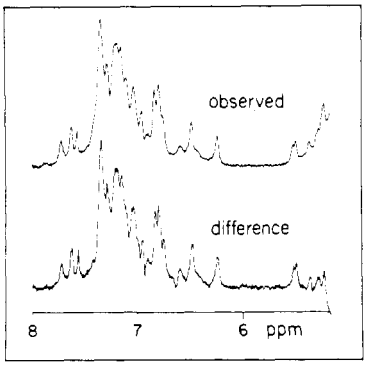

FIGURE 4: Comparison of the observed vs. difference spectra for the calmodulin–M13 complex. The top spectrum represents the observed spectrum of a 1:1 mixture of calmodulin and M13 and 1 mM Ca^{2+}. The bottom spectrum was generated by subtracting 0.6 times the spectral intensity of a normalized spectrum of isolated calmodulin from the spectrum of a 0.6:1 calmodulin:M13 mixture (third spectrum from bottom in Figure 3).

The hydrophobic drug trifluoperazine and a number of other small hydrophobic molecules have been widely used as models to study calmodulin interactions. Proton NMR studies of calmodulin–trifluoperazine interactions (Klevit et al., 1981; Dalgarno et al., 1984a) and calmodulin–W7 interactions (Klevit, 1981) indicate that although a number of calmodulin resonances are affected by drug binding, many are not. This is particularly significant considering that calmodulin binds at least two molecules of phenothiazine or naphthalenesulfonamide derivatives compared to one molecule of M13. Resonances affected by M13, but not by drugs, include the ring protons of Tyr-138 and the methyl protons of trimethyllysine-115, as well as a number of unassigned aromatic and aliphatic resonances.

The spectra in Figure 4 demonstrate that the system consisting of a mixture of calmodulin and M13 with calmodulin in molar excess is a two-component system in slow exchange.

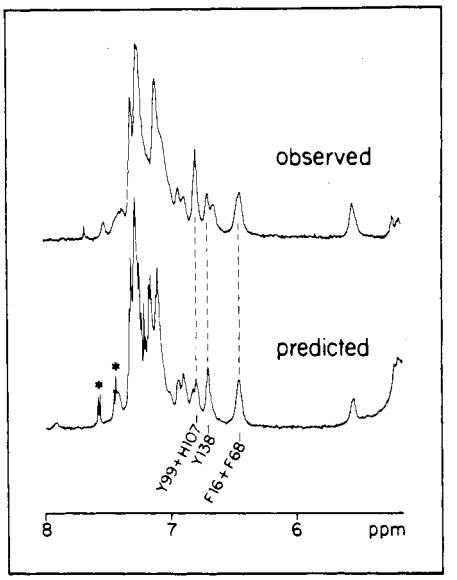

FIGURE 5: Predicted vs. observed spectra for M13 and calmodulin in EGTA. The bottom spectrum was generated by adding the separate spectra of calmodulin and M13 in EGTA. The top spectrum was obtained for an equimolar solution of M13 and calmodulin in 10 mM EGTA. The resonance assignments are from Dalgarno et al. (1984b). The dotted lines follow these resonances. Asterisks indicate tryptophanyl resonance from M13.

The top spectrum is that of an equimolar solution of calmodulin and M13. The bottom spectrum is a difference spectrum obtained by subtracting the spectrum of free calmodulin from the spectrum of calmodulin partially saturated with M13 ([CaM]:[M13] = 1.0:0.6). If the system is truly a two-component system, i.e., contains only free calmodulin and a single species of complex, the difference spectrum should be identical with that of the 1:1 complex. Indeed, the two spectra are virtually indistinguishable. Furthermore, no resonances that can be attributed to free M13 are observed in either spectrum.

The Ca^{2+} dependence of the interaction between M13 and calmodulin was investigated by adding EGTA (10 mM) to an equimolar solution of peptide and protein. The resulting spectrum (Figure 5) is very different from that obtained in the presence of Ca^{2+} (Figures 2–4), due in large part to the changes known to be effected by Ca^{2+} on the spectrum of calmodulin alone (Seamon, 1980). A predicted spectrum was generated by adding the spectra of isolated apocalmodulin (calmodulin in the absence of divalent metal ions) and isolated M13. On the whole, the predicted and observed spectra are quite similar. Major spectral features of the apocalmodulin spectrum, such as the chemical shift dispersion of the Ca^{2+}-binding loop C^{α}-proton peaks (4.8–5.5 ppm), the upfield-shifted aromatic peaks (6–7 ppm), the trimethyllysine-115 singlet (not shown), and the upfield-shifted methyl peaks (not shown), are unaffected by M13 in the presence of EGTA. The peak that contains the four ring proton resonances of Tyr-138 is split slightly. The change in the peak containing Tyr-99 and His-107 resonances is due to a slight difference in pH between the samples used to obtain the observed and predicted spectra. The most striking difference between the predicted and observed spectra is the increase in the line width of M13 resonances. This observation implies that there is an interaction between the peptide and calmodulin in the absence of free Ca^{2+}. Addition of high concentrations of NaCl results in a sharpening of the M13 resonances (unpublished observations), indicating that these Ca^{2+}-independent interactions are predominantly ionic. The overall similarity of the observed and predicted spectra indicates that in the absence of Ca^{2+}

neither calmodulin nor M13 undergoes major structural changes as a result of interaction. Ca^{2+}-independent interactions between calmodulin and M13 have previously been observed (Blumenthal et al., 1985), and because of the high concentrations of M13 and calmodulin used in both the CD (75 μM) and NMR (500 μM) experiments, some Ca^{2+}-independent interaction was predicted on the basis of considerations of free energy coupling (Olwin et al., 1984).

Discussion

Because of the many cellular processes that involve calmodulin–protein interactions, there has been much interest in the molecular aspects of calmodulin binding to various ligands. Early work showed that various hydrophobic ligands bind calmodulin in a Ca^{2+}-dependent manner (Weiss & Levin, 1978; La Porte et al., 1980; Hidaka et al., 1980), suggesting the importance of hydrophobic interactions in calmodulin–target protein interactions. Other work indicated the importance of ionic forces as well as other short-range effects in the interaction of calmodulin with target enzymes (Blumenthal & Stull, 1982). More recent work has indicated that the ability to form an amphipathic α-helix is an important structural determinant of certain model peptides that bind calmodulin with high affinity (Comte et al., 1983; Giedroc et al., 1983; Barnette et al., 1983; Malencik & Anderson, 1984; Cox et al., 1985; McDowell et al., 1985). Thus, a pattern of structural features expected to occur in the calmodulin-binding domain of a target enzyme has been developed. With the recent identification and sequence determination of the calmodulin-binding domain of skeletal muscle MLCK (Blumenthal et al., 1985; Takio et al., 1985; Edelman et al., 1985) the opportunity exists to compare the structure and modes of interaction of an authentic calmodulin-binding domain with those of the model ligands.

Investigation of the interaction of calmodulin with M13 by CD spectroscopy indicates that large increases in α-helical content are associated with complex formation. Most of the structural change is Ca^{2+}-dependent. Comparison of these data to CD data obtained by using model peptides such as endorphin (Giedroc et al., 1983), melittin (Maulet & Cox, 1983), and mastoparans (McDowell et al., 1985) and an amphiphilic model peptide (LK2) consisting only of leucine and lysine residues (Cox et al., 1985) is noteworthy in that although complex formation is associated with increased α-helical content in every case, the extent of increase is much greater in the case of M13. The α-helical contents of the peptides bound to calmodulin, calculated by assuming all of the observed ellipticity change is due to peptide, are 24, 50, 61, 71, and 101%, respectively, in the case of β-endorphin (Giedroc et al., 1983), the LK2 peptide (Cox et al., 1985), mastoparan X (McDowell et al., 1985), melittin (Maulet & Cox, 1983), and M13 (Table I). Of particular interest to the present study is the work of Mayr & Heilmeyer (1983), who studied the interaction of skeletal muscle MLCK and some of its proteolytic fragments with calmodulin using CD and found an increase in α-helical content upon complex formation. It is tempting to speculate that the calmodulin-binding domain of MLCK, which represents the extreme C-terminus of the enzyme (Takio et al., 1985; Edelman et al., 1985), may be largely unfolded when not complexed to calmodulin; then, following interaction with calmodulin, this region assumes an α-helical conformation which results in enzyme activation. This model is consistent with the observation that this region of MLCK is susceptible to limited proteolysis by trypsin and chymotrypsin (Edelman et al., 1985) and with data which indicate that hydrogen bonding (as would be associated with helix formation) is an important component of MLCK activation (Blumenthal & Stull, 1982).

The NMR spectra of mixtures of M13 and calmodulin in the presence of Ca^{2+} are quite different from the spectra of each molecule alone. While it was expected that many of the peptide resonances would be affected by complex formation, the dramatic changes observed in the calmodulin spectrum were not anticipated. There are two mechanisms through which the resonances of calmodulin can be affected by M13 binding. The first is through direct interaction between side chains, especially aromatic ones, resulting in ring-current shifts for the protons involved. If the spectral changes observed were due entirely to direct interactions, the effects should be localized to the contact regions of the molecule. The second mechanism is through indirect interactions resulting from conformational changes in the calmodulin molecule. The upfield-shifted aromatic resonances derived from two homologous aromatic clusters in each half of the calmodulin molecule [Phe-16, Phe-65, and Phe-68 in the N-terminal half and Phe-89, Phe-141, and Tyr-138 in the C-terminal half (Dalgarno et al., 1984b)] are specific resonances that are very sensitive to changes in camodulin tertiary structure and that are affected by M13 binding. The chemical shifts of these protons are indicative of the relative orientations of the first and fourth helices in each pair of Ca^{2+}-binding domains. Other assigned resonances affected by M13 binding include the methyl protons of trimethyllysine-115 (located between the two C-terminal Ca^{2+}-binding domains) and the downfield-shifted C^{α}-proton resonance, which is known to be derived from one of the N-terminal Ca^{2+}-binding loops (Dalgarno et al., 1984b). A large number of unassigned residues are also affected by M13 binding. In light of the evidence that only one molecule of peptide is bound in the complex and that this is bound in only one way, it seems unlikely that one 26-residue peptide could be responsible for all of the observed spectral changes through direct interaction alone. A more likely possibility is that M13 binding causes the observed changes in calmodulin's spectrum by directly interacting with specific amino acid side chains and by altering the tertiary structure of calmodulin.

Support for the idea that calmodulin undergoes changes in its tertiary structure as the result of binding M13 is provided by geometric considerations based on the recently reported three-dimensional structure of calmodulin (Babu et al., 1985). Even with the limited number of specific resonance assignments in the NMR spectrum of calmodulin, it is clear that the residues affected by M13 binding extend over most of the protein. For instance, trimethyllysine-115 lies at the extreme end of the molecule with the side chain directed toward the solvent. Approximately 60 Å away is the aromatic cluster composed of Phe-16, Phe-65, and Phe-68. If M13 is entirely α-helical, it would only span a distance of 39 Å. If, on the other hand, one assumes that only those residues predicted to form an α-helix do so and the rest are completely extended (i.e., 18 of 26 residues form a helix), the peptide could span approximately 55 Å. This length is almost that required to directly interact with the assigned residues at each end of the molecule; however, it is still difficult to account for the remainder of the observed spectral changes without invoking some changes in calmodulin tertiary structure. It is anticipated that as additional resonance assignments in the NMR spectrum of calmodulin are made, a more complete picture of the extent of M13's effect will emerge.

Changes in calmodulin structure induced by interaction with M13 suggest that target enzyme dependent conformational

changes in calmodulin could play an important role in the activation process. Until now, the accepted scheme for calmodulin's action included a conformational change upon Ca^{2+} binding but did not include any additional structural change in calmodulin upon interaction with a target enzyme. These target enzyme dependent structural changes may have importance in several different aspects of enzyme activation including maximizing binding interactions, conferring specificity, and/or effecting further conformational changes in the target enzyme necessary for expression of catalytic activity. Studies are currently in progress to determine the nature of these structural changes and their possible role in target enzyme interaction.

In summary, both calmodulin and M13 undergo significant spectral changes upon Ca^{2+}-dependent complex formation. These changes are different from spectral changes induced by other calmodulin-binding ligands. Work is now in progress to identify the M13 binding site on calmodulin, the residues in M13 involved in the interaction with calmodulin, and the structural features of both calmodulin and M13 in the complex. These studies should further our understanding of the molecular aspects of calmodulin action.

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