Structure of calmodulin–target peptide complexes

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The structures of the complexes between calcium-bound calmodulin and synthetic peptides comprising the calmodulin-binding domain of skeletal and smooth muscle myosin light-chain kinase have now been solved by NMR and X-ray crystallography, respectively. Within coordinate errors, there are no significant differences between the two structures. The two domains of calmodulin (residues 6–73 and 83–146) remain essentially unchanged upon complexation. The long central helix (residues 65–93) which connects the two domains in the crystal structure of calcium-bound calmodulin, however, is disrupted in the complex into two helices connected by a long flexible loop (residues 74–82), thereby enabling the two domains to clamp the bound peptide which adopts a helical conformation. The overall structure of the complex is globular, approximating an ellipsoid of dimensions 47 × 32 × 30 Å. The helical peptide is located in a hydrophobic channel which passes through the center of the ellipsoid at an angle of 45° to its long axis.

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Introduction

Calmodulin (CaM) is a ubiquitous Ca^{2+}-binding protein of 148 residues which is involved in a wide range of cellular Ca^{2+}-dependent signalling pathways, thereby regulating the activity of a large number of proteins including protein kinases, a protein phosphatase, nitric oxide synthase, inositol triphosphate kinase, NAD kinase, cyclic nucleotide phosphodiesterase, Ca^{2+} pumps and proteins involved in motility [1]. CaM-binding domains have been isolated from a number of CaM-dependent enzymes and have been shown to comprise peptide sequences with a high propensity for helix formation [1,2]. In addition, both circular dichroism [2,3] and NMR [4•,5] studies have shown that many naturally occurring CaM-binding peptides, such as mellitin and mastoporan, as well as synthetic peptides corresponding to CaM-binding domains, adopt a helical conformation upon binding to CaM.

The crystal structure of Ca^{2+}-CaM was solved a number of years ago [6–9]. It is a dumbbell-shaped molecule with an overall length of 65 Å which comprises two globular domains, each of which contains two Ca^{2+}-binding sites of the helix-loop-helix type, connected by a long, solvent-exposed, rigid central helix (residues 66–92) some eight-turns in length (Fig. 1). In solution, on the other hand, ^{1}H-^{15}N NMR relaxation measurements have demonstrated unambiguously that the central helix is disrupted near its mid-point, with residues 78–81 adopting an essentially unstructured 'random coil' conformation [10••]. Indeed, these residues are so flexible that the amino- and carboxy-terminal domains of Ca^{2+}-CaM effectively tumble independently of each other. Thus, in solution, the so-called 'central helix' is not a helix at all but is a 'flexible tether', whose purpose is to keep the two domains in close proximity for binding to their target [10••].

A range of biophysical studies, including cross-linking experiments [11] and small-angle X-ray and neutron scattering [12,13], have indicated that upon complexation with certain target peptides, CaM adopts a globular conformation. Ever since the appearance of the first Ca^{2+}-CaM crystal structure in 1985, the structural mechanism whereby Ca^{2+}-CaM recognizes its target sites has been the subject of considerable interest and debate, and a number of models for a Ca^{2+}-CaM–peptide complex have been proposed [14,15]. This problem has now been resolved as a result of the determination of the solution structure of a complex of Ca^{2+}-CaM with a target peptide (M13) from skeletal muscle myosin light-chain kinase (MLCK) [16••] using multi-dimensional heteronuclear-filtered and -edited NMR spectroscopy [17]. The solution structure was subsequently confirmed by the determination of the X-ray structure of a complex with a related peptide from smooth muscle MLCK [18].

In this review, we describe the structure and biological implications of the Ca^{2+}-CaM–peptide complexes determined by NMR and crystallography, the conformational changes that occur upon binding peptide, the factors stabilizing the peptide–protein interaction, and the relationship of the structures to biochemical data and previously proposed models.

Abbreviations

CaM—calmodulin; **MLCK**—myosin light-chain kinase; **NOE**—nuclear Overhauser effect; **rms**—root mean square.

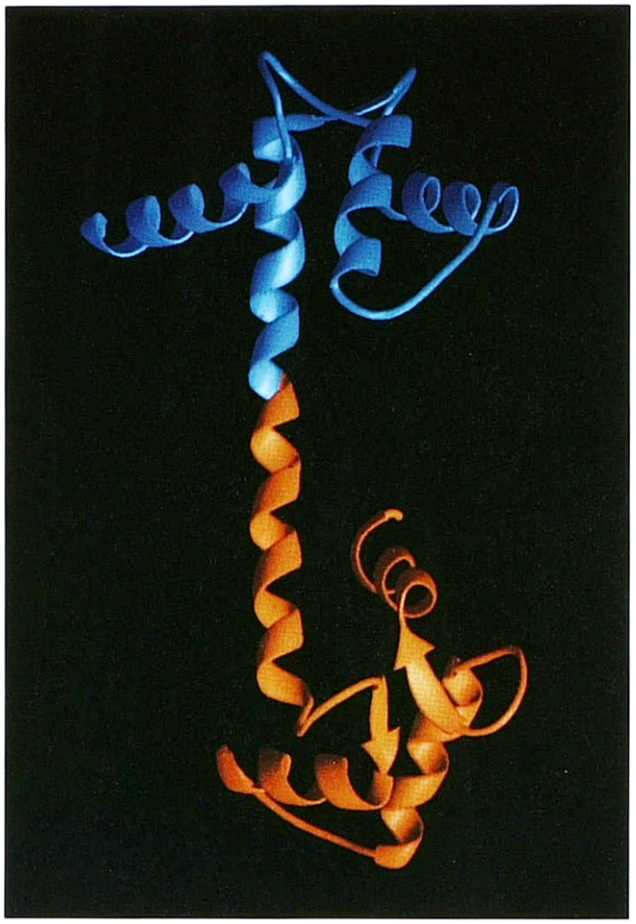

Fig. 1. Ribbon drawing of the X-ray structure of uncomplexed Ca^{2+}-CaM [6]. The amino terminal domain is shown in blue and the carboxy terminal one in red. The model was generated with the program RIBBONS (written by M Carson).

Solution structure of the Ca^{2+}-CaM–M13 complex

Structure determination of the complex

The 26-residue peptide M13 used for the solution studies comprises residues 577–602 of the CaM-binding domain of skeletal muscle MLCK. The solution structure was determined on the basis of 1995 experimental NMR restraints which included 133 interproton distance restraints between the peptide and the protein. The amino (residues 1–5) and carboxyl (residues 147–148) termini of CaM, the tether connecting the two domains of CaM (residues 74–82), and the amino (residues 1–2) and carboxyl (residues 22–26) termini of M13 were ill-defined by the NMR data and appear to be disordered in solution. The atomic root mean square (rms) distribution about the mean coordinate positions for the rest of the structure (i.e. residues 6–73 and 83–146 of CaM and residues 3–21 of M13) is 1.0 Å for the backbone atoms and 1.4 Å for all atoms. Thus, this structure represents a second-generation structure according to the classification of Clore and Gronenborn [17]. A stereoview showing a best-fit superposition of the 24 calculated structures is provided in Fig. 2.

Within the errors of the NMR coordinates, there is no significant conformational change within each domain (residues 4–74 and 82–146) between the complexed and uncomplexed states. Further, the overall conformation of the two domains is very similar, such that their backbone atoms can be superimposed with an atomic rms difference of 1.8 Å.

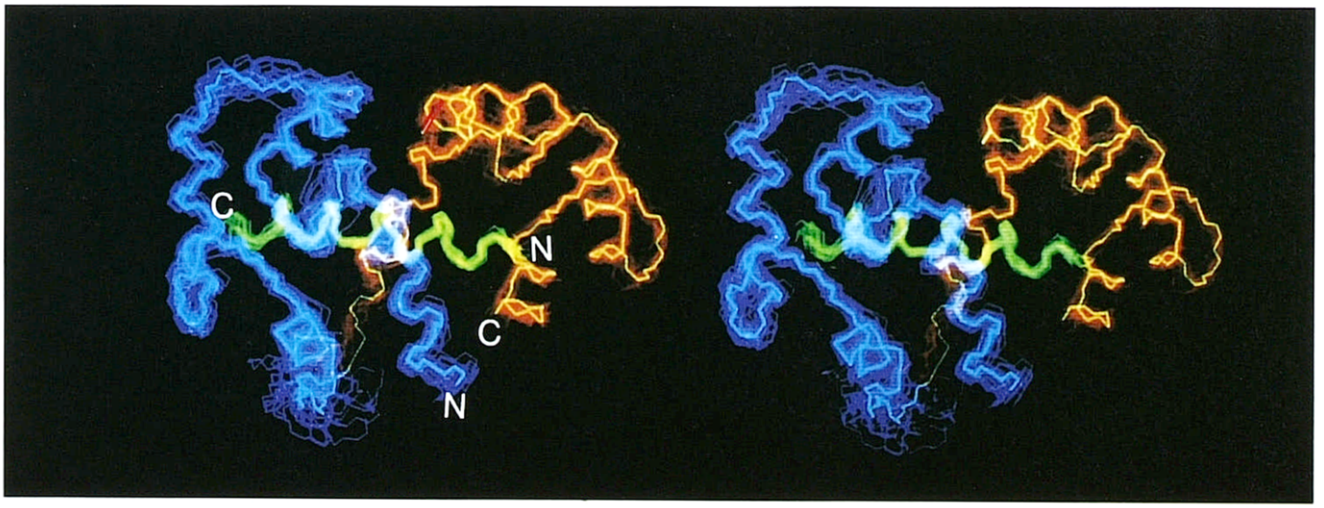

Fig. 2. Stereoview showing a best-fit superposition of the backbone (N, C_α, C) atoms of an ensemble of 24 calculated structures of the Ca^{2+}-CaM–M13 complex determined by NMR spectroscopy. The amino-terminal domain is shown in blue, the carboxy-terminal domain in red, and the M13 peptide in green; the restrained regularized mean structure is also included in the superposition and is highlighted. The coordinates were derived from [16••].

Conformational changes upon complexation

The major conformational change in Ca^{2+}-CaM that occurs upon binding to M13 involves an extension of the flexible tether (residues 78–81) in the middle of the 'central' helix of the solution structure of free Ca^{2+}-CaM [10•] to a long flexible loop extending from residues 74–81, flanked by two helices (residues 65–73 and 83–93), thereby enabling the two domains to come together to grip the peptide rather like two hands capturing a rope (Fig. 3). The hydrophobic channel formed by the two domains is complementary in shape to that of the peptide helix. This is clearly illustrated by the schematic ribbon drawings shown in Fig. 4, which also highlights the approximate twofold pseudo-symmetry of the complex. Thus, whereas the two domains of CaM are arranged in an approximately orthogonal manner to each other in the crystal structure of Ca^{2+}-CaM [6–9], in the Ca^{2+}-CaM–M13 complex they are almost symmetrically related by a 180° rotation about a twofold axis. A large conformational change also occurs in the M13 peptide upon complexation from a random-coil state to a well defined helical conformation. Indeed, the helix involves all the residues (3–21) of M13 that interact with CaM, whereas the amino (residues 1–2) and carboxyl (residues 22–26) termini of the peptide, which do not interact with CaM, remain disordered.

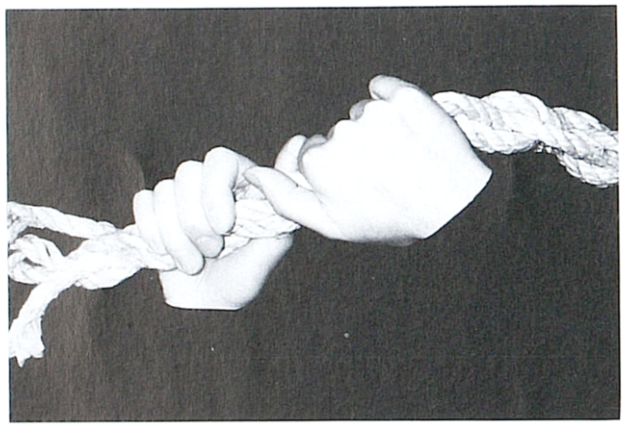

Fig. 3. Schematic representation of the structure of the Ca^{2+}-CaM–peptide complex, in which the peptide is represented by a rope, and the two domains of CaM by two right hands.

Complexation results in a decrease in the solvent-accessible surface area of CaM and M13 of 1848 and 1477 Å^2, respectively, which corresponds to a decrease in the calculated solvation free energy of folding [19] of 18 and 20 kcal mol^{-1}, respectively. This large decrease in solvation free energy can account for the very tight binding ($K_a = 10^9$ M^{-1}) of M13 to CaM. In addition, the accessible surface area of the portion of M13 (residues 3–21) in direct contact with CaM in the complex is only 494 Å^2, compared with an accessible surface area of 3123 Å^2 for a random coil and 2250 Å^2 for a helix. Thus, over 80% of the surface of the peptide in contact with CaM is buried.

In Figs 2 and 4, the roof of the channel is formed by helices II (residues 29–38) and VI (residues 102–111) of the amino- and carboxy-terminal domains, respectively, which run antiparallel to each other, and the floor is formed by the flexible loop (residues 74–82) connecting the two domains and by helix VIII (residues 138–146) of the carboxy-terminal domain. The front of the channel in Fig. 4(a) and the left wall of the channel in Fig. 4(b) is formed by helices I (residues 7–19) and IV (residues 65–73) and the mini-antiparallel β-sheet comprising residues 26–28 and 62–64, all derived from the amino-terminal domain; the back of the channel in Fig. 4(a) and the right wall of the channel in Fig. 4(b) is formed by helices V (residues 83–93) and VIII (residues 138–146) and the mini-antiparallel β-sheet comprising residues 99–101 and 135–137, all from the carboxy-terminal domain. The two domains of CaM are staggered with a small degree of overlap, such that the hydrophobic face of the amino-terminal domain mainly contacts the carboxy-terminal half of the M13 peptide, whereas the carboxy-terminal domain principally interacts with the amino-terminal half of M13 (Fig. 4a).

The overall Ca^{2+}-CaM–M13 complex has a compact globular shape approximating to an ellipsoid with dimensions 47×32×30 Å. The helical M13 peptide passes through the center of the ellipsoid at an angle of 45° to its long axis. By way of contrast, the approximate dimensions of the Ca^{2+}-CaM X-ray structure are 65×30×30 Å [6–9]. This change in dimensions is reflected by a change in the ratio of the three principal components of the inertia tensor from 1.00 : 0.22 : 1.07 for the X-ray structure of Ca^{2+}-CaM to 1.00 : 1.44 : 1.58 for the solution structure of the Ca^{2+}-CaM–M13 complex. In addition, the calculated radius of gyration for Ca^{2+}-CaM–M13 is 17 Å, which is completely consistent with the decrease in the radius of gyration from 21 to 16 Å observed by both small-angle X-ray and neutron scattering upon complexation of Ca^{2+}-CaM with M13 [13].

Interactions stabilizing the complex

The Ca^{2+}-CaM–M13 complex is stabilized by numerous hydrophobic interactions (summarized in Fig. 5). Particularly striking are the interactions of Trp4 and Phe17 of the peptide which serve to anchor the amino- and carboxy-terminal halves of M13 to the carboxy-terminal and amino-terminal hydrophobic patches of CaM, respectively (Fig. 4b). These interactions also involve a large number of methionine residues, which are unusually abundant in CaM, in particular four methionines in the carboxy-terminal domain (Met109, Met124, Met144 and Met145) and three in the amino-terminal domain (Met36, Met51 and Met71). As methionine is an unbranched hydrophobic residue extending over four heavy atoms (C_β, C_γ, S_δ and C_ε), the abundance of methionines can generate a hydrophobic surface whose detailed topology is readily adjusted by minor changes in side-chain conformation, thereby providing

a mechanism for the accommodation and recognition of different bound peptides [15].

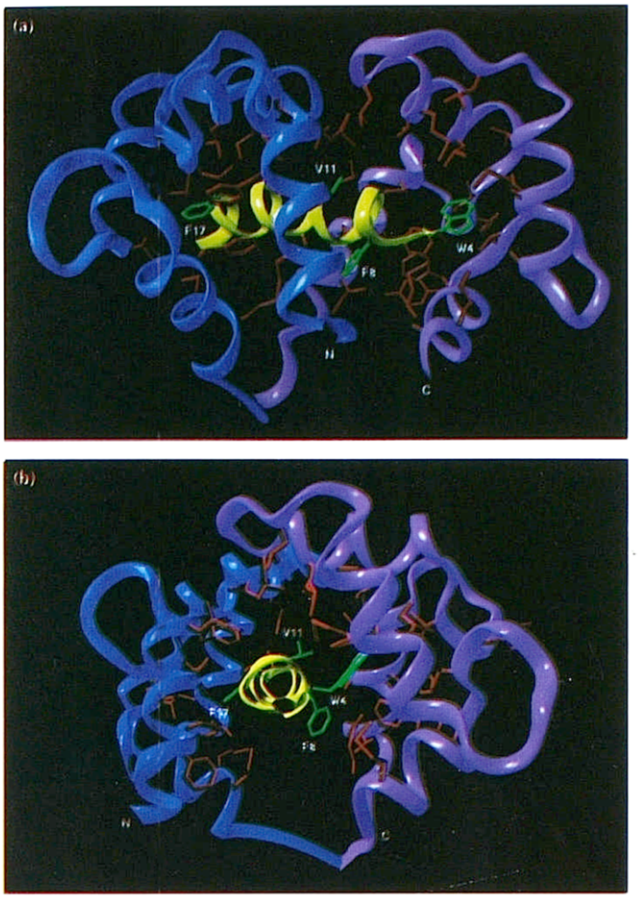

Fig. 4. (a,b) Two orthogonal views of a schematic ribbon drawing representation of the solution NMR structure of the Ca^{2+}-CaM–M13 complex, with the amino-terminal domain in blue, the carboxy-terminal domain in purple, and the M13 peptide in yellow. The hydrophobic side chains of the amino- and carboxy-terminal domains of CaM are shown in red, and the Trp4, Phe8, Val11 and Phe17 side chains of the peptide are displayed in green. The model was generated using the program VISP (written by E de Castro and S Edelstein), and the coordinates were taken from [16••].

In addition to hydrophobic interactions, a number of possible electrostatic interactions can be deduced from the calculated NMR structures. Putative interactions exist between the arginine and lysine residues of M13 and the glutamate residues of CaM (also included in Fig. 5): Glu11 and Glu14 in helix I lie within 5 Å of Lys5 and Lys6 of M13; Glu83, Glu84 and Glu87 in helix V of CaM are close to Lys19, Arg16 and Lys18 of M13, respectively; and Glu127 in helix VII of CaM is close to Arg3 of M13.

Crystal structure of the Ca^{2+}-CaM complex with a target peptide from smooth muscle myosin light-chain kinase

After the determination of the solution structure of the complex with a peptide from skeletal muscle MLCK, a crystal structure with a related 20-residue peptide from the CaM-binding domain of smooth muscle MLCK was solved at a resolution of 2.4 Å [18]. The sequence identity between the peptides used for the NMR and crystallographic studies is 30%. As expected, the overall structure and the interactions between the peptide and the protein are very similar. The backbone rms difference between the solution and X-ray structures for the amino- (residues 6–73) and carboxy- (residues 83–146) terminal domains, for the peptide (residues 3–19 in the numbering scheme for M13) and for the whole complex excluding disordered regions (i.e. for residues 6–73 and 83–146 of CaM and residues 3–19 of the peptide) are 1.9, 1.4, 1.1 and 1.9 Å, respectively. The precision of the NMR coordinates is ~1 Å for the backbone atoms (which translates to an accuracy of ~1.6 Å) [20•]. At a resolution of 2.4 Å, the accuracy of the X-ray coordinates is ~0.4–0.5 Å [21]. Thus, one can conclude that there is no significant difference between the solution and X-ray structures within the errors of the coordinates.

There are a few clear-cut minor differences, however, in the linker region between the solution and X-ray structures of the complex. Thus, in solution, residues 74–81 are disordered on the basis of the nuclear Overhauser effect (NOE) data [16••], as well as the $^{13}C_\alpha$ and $^{13}C_\beta$ chemical shifts [22]. In contrast, residues 73–77 in the crystal structure have an extended conformation with well defined helices proceeding and following this segment [18]. This minor difference results presumably from crystal packing forces and/or a scarcity of NOE data in this region.

Correlation with biochemical data

The solution and X-ray structures of the Ca^{2+}-CaM–peptide complexes explain a number of interesting observations. Studies of backbone amide-exchange behavior have shown that upon complexation with M13, the amide-exchange rates of residues 75–79 are increased substantially [23]. Previous NMR studies on Ca^{2+}-CaM indicated that the long central helix is already disrupted near its middle (between Asp78 and Ser81) in solution [22] and that large variations in the orientation of one domain relative to the other occur randomly with time [10••]. The further disruption of the central helix upon complexation seen in the structures of the two complexes is manifested by the increased amide-exchange rates, and supports the view of the central helix serving as a flexible linker between the two domains. Similarly, the structures of the two complexes explain the finding that as many as four residues can be deleted from the middle of the central helix without dramatically altering the stability or shape of the Ca^{2+}-CaM–M13 complex [24,25], as the long flexible loop connecting the two domains can readily be shortened without causing any alteration in the structure (cf. Fig. 4).

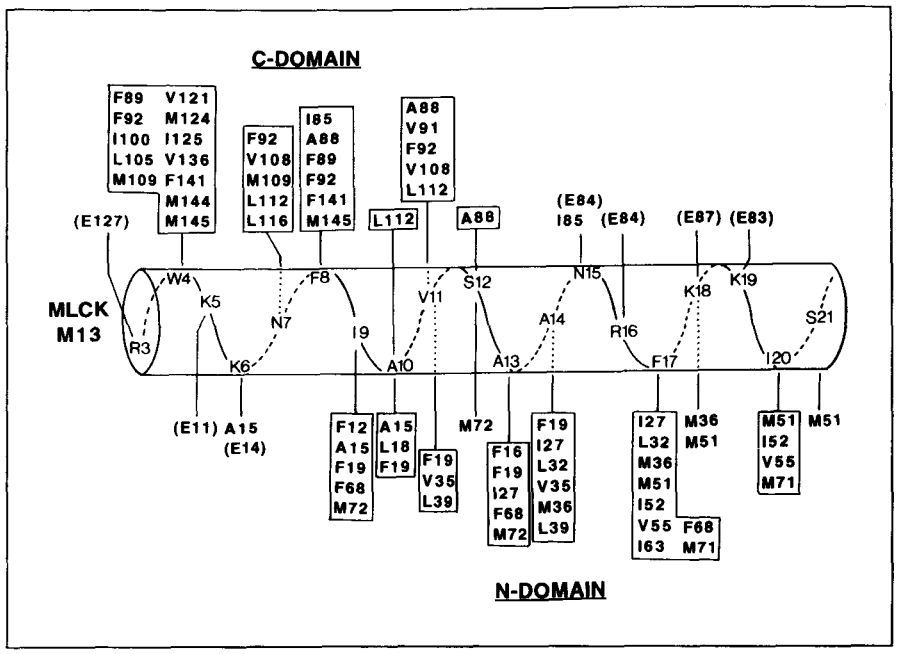

Fig. 5. Summary of residue pairs for which intermolecular NOEs between CaM and M13 are observed. CaM residues involved in hydrophobic interactions are boxed. Also included are potential electrostatic interactions between negatively charged glutamate residues of CaM, shown in parentheses, and positively charged lysine and arginine residues of M13; it should be noted that the electrostatic interactions have been inferred purely from the structure as no NOEs have been identified between these pairs of residues. Adapted from [16••].

The observation from photoaffinity-labeling studies that the two domains of CaM interact simultaneously with opposite ends of the peptide, such that residue 4 of the peptide (numbering for M13) can be cross-linked to Met124 or Met144 of the carboxy-terminal domain and that residue 13 of the peptide can be cross-linked to Met71 of the amino-terminal domain [15,26], is in agreement with the structural finding that the amino-terminal half of the peptide interacts predominantly with the carboxy-terminal domain whereas the carboxy-terminal half of the peptide interacts predominantly with the amino-terminal domain (Figs 2–4). The observation that at least 17 residues of the M13 peptide from either skeletal muscle or smooth muscle are necessary for high-affinity binding [27,28] is readily explained by the intimate interactions of the carboxy-terminal hydrophobic residue (i.e. Phe17) with the amino-terminal domain of CaM by which the peptide is anchored. Finally, the structure accounts for experiments in which cross-linking of residues 3 and 146 of CaM, mutated to cysteine, has no effect on the activation of MLCK, even if the central helix is cleaved proteolytically at Lys77 by trypsin [11]. Whereas the C_αs of residues 3 and 146 are 37 Å apart in the X-ray structure of Ca^{2+}-CaM, they are only 20 Å apart in the solution structure of the Ca^{2+}-CaM–M13 complex, which is close enough to permit cross-linking to occur.

Comparison with previous models

It is of interest to compare the structures of the Ca^{2+}-CaM–peptide complexes with the class III model proposed by Persechini and Kretsinger [14]. In this model, binding is accomplished by introducing a kink in the central helix by simply changing the ϕ,ψ angles of Ser81 from –57°,–47° to –54°,+98°. The general features of the structure, namely the two domains coming together in close proximity to form a hydrophobic channel occupied by a peptide in a helical conformation, are similar in the two cases.

There are however substantial differences. First, the extent of the M13 peptide in direct contact with CaM is a little greater in the model (residues 1–21) than in the solution structure (residues 3–21). Second, the peptide in the model is oriented at 180° relative to that in the solution and X-ray structures, such that the amino- and carboxy-terminal halves of the peptide in the model interact with the amino- and carboxy-terminal domains of CaM, respectively. (The possibility of inverting the orientation of the peptide is however stated in the description of the model.) Third, the relative orientation of the two domains is significantly different. This can be attributed to the fact that the central helix remains essentially intact in the model except for the kink introduced at Ser81, whereas in the solution structure it is broken up and partitioned into two helices separated by a long eight-residue flexible loop, thereby permitting a more optimal arrangement of the two domains. Thus, when the carboxy-terminal domains of the model and solution structures are superimposed, the atomic rms displacement between the amino-terminal domains of the two structures is 8 Å. As a consequence, the width of the channel is a little wider and the two domains are slightly more separated in the model than in the solution structure.

An alternative model was also proposed by O'Neil and DeGrado [15]. As in the previous model, a kink was introduced by altering the ϕ,ψ angles of Ser81. Although the orientation of the peptide in this model, based on the results of photo-labeling studies, was correct, only a relatively small distortion was introduced into the central helix, so that the complex retained to some degree a dumbbell shape as opposed to the globular

shape observed in the actual structure, and did not possess a well defined hydrophobic channel.

Implications of the structures of the calmodulin–target peptide complexes

A large body of experimental data shows that CaM binds to numerous proteins whose binding domains exhibit a propensity for α-helix formation [1,2]. A comparison of these sequences reveals little homology. Yet many of the very tightly binding peptides ($K_a \geq 5 \times 10^7 M^{-1}$) share the common property of containing either aromatic residues or long-chain hydrophobic residues (leucine, isoleucine or valine) separated by 12 residues, as summarized in Fig. 6. In the case of M13, these two residues are Trp4 and Phe17, which are exclusively in contact with the carboxy- and amino-terminal domains of CaM, respectively (Figs 4 and 5). Given that these two residues are involved in more hydrophobic interactions with CaM than any other residues of the peptide (cf. Fig. 5), it seems likely that this feature of the sequence can be used to align the CaM-binding sequences listed in Fig. 6, thereby permitting one to predict their interaction with CaM.

It is clear from this alignment that the pattern of hydrophobic and hydrophilic residues is in general comparable for the various peptides, suggesting that the mode of binding and the structure of the corresponding complexes with Ca^{2+}-CaM are also likely to be similar. For example, there is, in general, conservation of hydrophobic residues at the positions equivalent to Phe8 (in M13) which interacts with the carboxy-terminal domain and Val11 which interacts with both domains (cf. Figs 4 and 5). In addition, there are no acidic residues present which would result in unfavorable electrostatic interactions with the negatively charged glutamate residues on the surface of CaM (cf. Fig. 5). The minimum length of peptide required for high-affinity binding to Ca^{2+}-CaM is defined by the 14-residue mastaporans, which comprise the two hydrophobic residues at the amino and carboxyl termini (Fig. 6) and have approximately the same equilibrium association constant ($K_a = 1–3 \times 10^9 M^{-1}$) as M13 [3]. This structural alignment also predicts that a peptide stopping just short of the second hydrophobic residue of the pair (i.e. the residue equivalent to Phe17) would only bind to the carboxy-terminal domain and that the resulting complex would therefore retain the dumbbell shape of Ca^{2+}-CaM.

This is exactly what has been observed by small-angle X-ray scattering using two synthetic peptides, C24W and C20W (Fig. 6), comprising different portions of the CaM-binding domain of the plasma membrane Ca^{2+} pump [29]. The complex with the C24W peptide, which corresponds to residues 1–24 of M13 and contains a tryptophan at position 4 and a valine at position 17, has a globular shape similar to that of Ca^{2+}-CaM–M13. The complex with the C20W peptide, on the other hand, which corresponds to residues 4–16 of M13 and therefore lacks the carboxy-terminal hydrophobic residue of the pair, retains the dumbbell shape of Ca^{2+}-CaM [29], suggesting that the peptide only binds to the carboxy-terminal domain.

Concluding remarks

The solution [16••] and X-ray [18] structures of the two complexes of Ca^{2+}-CaM with target peptides from skeletal and smooth muscle MLCK reveal an unusual binding mode in which the target peptide is sequestered into a hydrophobic channel formed by the two domains of CaM with interactions involving 19

	1					5					10					15					20					25				
SK-MLCK M13				K	R	R	W	K	K	N	F	I	A	V	S	A	A	N	R	F	K	K	I	S	S	S	G	A	L	M
SM-MLCK M13				R	R	K	W	Q	K	T	G	H	A	V	R	A	I	G	R	L	S	S	S			.				
Ca Pump C24W				Q	I	L	W	F	R	G	L	N	R	I	Q	T	Q	I	R	V	V	N	A	F	R	S	S			
C20W	L	R	R	G	Q	I	L	W	F	R	G	L	N	R	I	Q	T	Q	I	K										
Calspermin			A	R	R	K	L	K	A	A	V	K	A	V	V	A	S	S	R	L	G	S								
Calcineurin		A	R	K	E	V	I	R	W	K	I	R	A	I	G	K	M	A	R	V	S	F	V	L						
Mastaporan							I	N	L	K	A	L	A	A	L	A	K	K	I	L										
Mastaporan X							I	N	W	K	G	I	A	A	M	A	K	K	L	L										
Mellitin		G	I	G	A	V	L	K	V	L	T	T	G	L	P	A	L	I	S	W	I	K	R	K	R	Q	Q		
Interacting							C				C			C					N										
domain of CaM														N															

Fig. 6. Alignment of tightly binding ($K_a > 5 \times 10^7 M^{-1}$) CaM-binding sequences based on the structural role of Trp4 and Phe17 in anchoring the M13 peptide to the carboxy- and amino-terminal domains of CaM, respectively. Each sequence features a pair of aromatic and/or long-chain aliphatic residues (boxed) separated by a stretch of 12 residues which correspond to Trp4 and Phe17 of M13. In addition, there are generally hydrophobic residues at positions equivalent to Phe8 (which interacts with the carboxy-terminal domain) and Val11 (which interacts with both domains). Adapted from [16••].

residues of the target peptide (i.e. residues 3–21 of M13). In addition, a key requirement appears to be the presence of two long-chain hydrophobic or aromatic residues separated by 12 residues in order to anchor the peptide to the two domains of CaM (Fig. 4). By anology, the rope (i.e. the CaM-binding domain of the target) has to be long enough and have two knots at each end for the two hands (i.e. domains) of CaM to grip it (Fig. 3). This particular mode of binding is therefore only likely to occur if the CaM-binding site is located either at an easily accessible carboxyl or amino terminus or in a long exposed surface loop of the target protein. An example of the former is MLCK and of the latter is calcineurin. In accordance with their location, the CaM-binding sites in these proteins are susceptible to proteolysis [28,30].

Clearly, other types of complexes between Ca^{2+}-CaM and its target proteins are possible given the inherent flexibility of the central helix [10••,22,23]. For example, in the case of the γ-subunit of phosphorylase kinase, it appears that there are two discontinuous CaM-binding sites which are capable of binding to Ca^{2+}-CaM simultaneously [31], and binding of a peptide derived from one of these sites causes elongation rather than contraction of Ca^{2+}-CaM [32], indicating that the complex is of a quite different structural nature. Similarly, in the case of cyclic nucleotide phosphodiesterase [33] and CaM kinase II [34], the CaM-binding sequences do not have the same spacing of hydrophobic residues seen in M13 and the other sequences listed in Fig. 6, and, in addition, CaM kinase II is not susceptible to proteolysis in the absence of phosphorylation [35], suggesting that the mode of binding is different again. Thus, in all likelihood, the complexes of Ca^{2+}-CaM with target peptides from skeletal and smooth MLCK represent one of a range of Ca^{2+}-CaM binding modes that achieve CaM–target protein interactions in an efficient and elegant manner.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. COHEN P, KLEE CB (EDS): *Molecular Aspects of Cellular Recognition*, vol 5. New York: Elsevier; 1988.

2. DEGRADO WF: Design of Peptides and Proteins. *Adv Protein Chem* 1988, 39:51–125.

3. COX JA, COMTE M, FITTON JE, DEGRADO WF: The Interaction of Calmodulin with Amphiphilic Peptides. *J Biol Chem* 1985, 260:2527–2534.

4. • IKURA M, BAX A: Isotope Filtered 2D NMR of a Protein-Peptide Complex: Study of a Skeletal Muscle Myosin Light Chain Kinase Fragment Bound to Calmodulin. *J Am Chem Soc* 1992, 114:2433–2440.
The peptide corresponding to the CaM-binding domain of smooth muscle MLCK is shown to adopt a helical conformation upon complexation with CaM using unlabeled peptide and $^{13}C/^{15}N$-labeled CaM, and ^{12}C- and ^{15}N-filtered NMR experiments.

5. ROTH SM, SCHNEIDER DM, STROBEL LA, VANBERKUM MFA, MEANS AR, WAND AJ: Structure of Smooth Muscle Myosin Light-Chain Kinase Calmodulin-Binding Domain Peptide Bound to Calmodulin. *Biochemistry* 1991, 30:10078–10084.

6. BABU YS, SACK JS, GREENHOUGH TJ, BUGG CE, MEANS AR, COOK WJ: Three Dimensional Structure of Calmodulin. *Nature* 1985, 315:37–40.

7. KRETSINGER RH, RUDNICK SE, WEISSMAN LJ: Crystal Structure of Calmodulin. *J Inorg Biochem* 1986, 28:289–302.

8. BABU YS, BUGG CE, COOK WJ: Structure of Calmodulin Refined at 2.2 Å Resolution. *J Mol Biol* 1988, 204:191–204.

9. TAYLOR DA, SACK JS, MAUNE JF, BECKINGHAM K, QUIOCHO FA: Structure of Recombinant Calmodulin from *Drosophila melanogaster* Refined at 2.2 Å Resolution. *J Biol Chem* 1991, 266:21375–21380.

10. •• BARBATO G, IKURA M, KAY L, PASTOR RW, BAX A: Backbone Dynamics of Calmodulin Studied by ^{15}N Relaxation Using Inverse Detected Two-Dimensional NMR Spectroscopy: the Central Helix is Flexible. *Biochemistry* 1992, 31:5269–5278.
The first unequivocal demonstration that the so-called central helix connecting the two domains of calmodulin is highly mobile and acts as a flexible linker, thereby permitting the two domains to tumble essentially independently of each other in solution.

11. PERSECHINI A, KRETSINGER RH: The Central Helix of Calmodulin Functions as a Flexible Tether. *J Biol Chem* 1988, 263:12175–12178.

12. KATAOKA M, HEAD JF, SEATON BA, ENGELMAN DM: Mellitin Binding Causes a Large Calcium Dependent Conformational Change in Calmodulin. *Proc Natl Acad Sci USA* 1989, 86:6944–6948.

13. HEIDORN DB, SEEGER PA, ROBKOP SE, BLUMENTHAL DK, MEANS AR, CRESPI H, TREWHELLA J: Changes in the Structure of Calmodulin Induced by a Peptide Based on the Calmodulin-Binding Domain of Myosin Light Chain Kinase. *Biochemistry* 1989, 28:6757–6764.

14. PERSECHINI A, KRETSINGER RJ: Toward a Model of the Calmodulin-Myosin Light Chain Kinase Complex: Implications for Calmodulin Function. *J Cardiovas Pharmacol* 1998, 12 (suppl 5):1–12.

15. O'NEIL KT, DEGRADO WF: How Calmodulin Binds Its Targets: Sequence Independent Recognition of Amphiphilic α-Helices. *Trends Biochem Sci* 1990, 15:59–64.

16. •• IKURA M, CLORE GM, GRONENBORN AM, ZHU G, KLEE CB, BAX A: Solution Structure of a Calmodulin-Target Peptide Complex by Multidimensional NMR. *Science* 1992, 256:632–638.
This paper describes the first structure determination of a CaM–target peptide complex using state-of-the-art multidimensional heteronuclear-filtered and -edited NMR experiments.

17. CLORE GM, GRONENBORN AM: Structures of Larger Proteins in Solution: Three- and Four-Dimensional Heteronuclear NMR Spectroscopy. *Science* 1991, 252:1390–1399.

18. MEADOR WE, MEANS AR, QUIOCHO FA: Target Enzyme Recognition by Calmodulin: 2.4 Å Structure of a Calmodulin-Peptide Complex. *Science* 1992, 257:1251–1255.

19. EISENBERG D, MCLAGHLAN AD: Solvation Energy in Protein Folding and Binding. *Nature* 1986, 319:199–203.

20. CLORE GM, ROBIEN MA, GRONENBORN AM: **Exploring the Limits of Precision and Accuracy of Protein Structures Determined by Nuclear Magnetic Resonance Spectroscopy.** *J Mol Biol* 1993, 231:82–102.

A detailed discussion of the factors affecting both the precision and accuracy of NMR structures and the relationship between precision and accuracy.

21. CLORE GM, GRONENBORN AM: **Comparison of the Solution Nuclear Magnetic Resonance and X-Ray Crystal Structures of Human Recombinant Interleukin-1β.** *J Mol Biol* 1991, 221:47–53.

22. IKURA M, KAY LE, KRINKS M, BAX A: **Triple-Resonance Multidimensional NMR Study of Calmodulin Complexed with the Binding Domain of Skeletal Muscle Myosin Light-Chain Kinase: Indication of a Conformational Change in the Central Helix.** *Biochemistry* 1991, 30:5498–5504.

23. SPERA S, IKURA M, BAX A: **Measurement of the Exchange Rates of Rapidly Exchanging Amide Protons: Application to the Study of Calmodulin and Its Complex with a Myosin Light Chain Kinase Fragment.** *J Biomol NMR* 1991, 1:155–165.

24. PERSECHINI A, BLUMENTHAL DK, JARRETT HW, KLEE CB, HARDY DO, KRETSINGER RH: **The Effects of Deletions in the Central Helix of Calmodulin on Enzyme Activation and Peptide Binding.** *J Biol Chem* 1989, 264:8052–8058.

25. KATAOKA M, HEAD JF, PERSECHINI A, KRETSINGER RH, ENGELMAN DM: **Small-Angle X-Ray Scattering Studies of Calmodulin Mutants with Deletions in the Linker Region of the Central Helix Indicate that the Linker Region Retains Predominantly α-Helical Conformation.** *Biochemistry* 1991, 30:1188–1192.

26. O'NEIL KT, ERICKSON-VITTANEN S, DEGRADO WF: **Photolabeling of Calmodulin with Basic, Amphilic α-Helical Peptides Containing *p*-Benzoylphenylalanine.** *J Biol Chem* 1989, 264:14571–14578.

27. LUKAS TJ, BURGESS WH, PREDERGAST FG, LAU W, WATTERSON DM: **Calmodulin Binding Domains: Characterization of a Phosphorylation and Calmodulin Binding Site from Myosin Light Chain Kinase.** *Biochemistry* 1986, 25:1458–1464.

28. BLUMENTHAL DK, KREBS EG: **Preparation and Properties of the Calmodulin Binding Domain of Skeletal Muscle Myosin Light Chain Kinase.** *Methods Enzymol* 1987, 139:115–126.

29. KATAOKA M, HEAD JF, VORHERR T, KREBS J, CARAFOLI E: **Small-Angle X-Ray Scattering Study of Calmodulin Bound to Two Peptides Corresponding to Parts of the Calmodulin-Binding Domain of the Plasma Membrane Ca^{2+} Pump.** *Biochemistry* 1991, 30:6247–6251.

30. GUERINI D, KLEE CB: **Structural Diversity of Calcineurin, Ca^{2+}-Calmodulin Stimulated Phosphatases.** *Adv Protein Phosphatases* 1991, 6:391–410.

31. DASGUPTA M, HONEYCUTT T, BLUMENTHAL DK: **The γ Subunit of Skeletal Muscle Phosphorylase Kinase Contains Two Noncontiguous Domains that Act in Concert to Bind Calmodulin.** *J Biol Chem* 1989, 264:17156–17163.

32. TREWHELLA J, BLUMENTHAL DK, ROKOP SE, SEEGER PA: **Small-Angle Scattering Studies Show Distinct Conformations of Calmodulin in Its Complex with Two Peptides Based on the Regulatory Domain of the Catalytic Subunit of Phosphorylase Kinase.** *Biochemistry* 1990, 29:9316–9324.

33. CHARBONNEAU H, KUMAR S, NOVACK JP, BLUMENTHAL DK, GRIFFIN PR, SHABANOWITZ J, HUNT DF, BEAVO JA, WALSH KA: **Evidence for Domain Organization within the 61-kDa Calmodulin-Dependent Cyclic Nucleotide Phosphodiesterase from Bovine Brain.** *Biochemistry* 1991, 30:7931–7940.

34. BENNETT MK, KENNEDY MB: **Deduced Primary Structure of the β Subunit of Brain Type II Ca^{2+}/Calmodulin Dependent Protein Kinase Determined by Molecular Cloning.** *Proc Natl Acad Sci USA* 1987, 84:1794–1796.

35. KWIATKOWSKI AP, KING MM: **Autophosphorylation of the Type II Calmodulin Dependent Protein Kinase is Essential for the Formation of a Proteolytic Fragment with Catalytic Activity: Implications for Long-Term Synaptic Potentiation.** *Biochemistry* 1989, 28:5380–5385.

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