3.4: Calmodulin

Calmodulin is a small acidic protein ($M_r \approx 16{,}700$), the amino-acid sequence of which has been remarkably preserved during evolution. Early on, an analysis of its amino-acid sequence indicated that it should have four Ca^{2+}-binding sites, a deduction that proved to be correct. The three-dimensional x-ray structure of bovine brain calmodulin[85] has been solved to a resolution of 2.2 Å. A space-filling model is shown in Figure 3.17. (See color plate section, page C-9.) The molecule has a dumbbell-like shape, with two globular domains connected by an eight-turn α-helix—an unusual structural feature. In the crystal structure, there are no direct contacts between the two globular domains, each of which contains two Ca^{2+}-binding sites. The Ca^{2+}sites are all constructed in the same way: two α-helices separated by a calcium-binding loop, 12 amino acids long, and wrapped around the Ca^{2+} ion. This structural arrangement is nearly identical with that first observed in the x-ray structure of carp parvalbumin, and is colloquially termed "*the EF-hand*."[86] This structural unit is also observed in all available x-ray structures of proteins of the calmodulin superfamily (see Sections V.B and V.C). The Ca^{2+} ligands are all oxygen atoms, located approximately at the vertices of a pentagonal bipyramid.

The binding of Ca^{2+} and other cations to CaM has been extensively investigated.[87] The first two Ca^{2+} ions are bound in a cooperative manner, with an average binding constant of about 2×10^5 M^{-1} in 150 mM KCl and 1 mM Mg^{2+}. The third and fourth Ca^{2+} ions are bound with binding constants of about 3×10^4 M^{-1} under the same conditions. Spectroscopic evidence has shown that the first two Ca^{2+}-ions are bound in the C-terminal domain. Mg^{2+} has been shown to bind primarily to the N-terminal domain (see Table 3.2).[88]

The rates of dissociation of Ca $^{2+}$ from the $(Ca^{2+})_4$ CaM complex have been studied by both stopped-flow and NMR techniques.[89,90] Fast and slow processes are observed, both corresponding to the release of two Ca^{2+} ions. At an ionic strength I = 0.1 and 25 °C, the rates for the two processes differ by a factor of 30 (see Table 3.4).

Table 3.4 - Rates of Ca^{2+}-dissociation and -association of some enzymes and proenzymes.

	k_{off} [s^{-1}]	k_{on} [M^{-1} s^{-1}]
Macrobicyclic amino cryptate [2.2.2]	0.3	10^4
Phospholipase A2	1.1×10^3	4×10^6
sTroponin C: Ca^{2+} sites	300	
sTroponin C: Ca^{2+}-Mg^{2+} sites	5	
Trypsin	3	1.1×10^5
Trypsinogen	≤ 10	6×10^4
Chymotrypsin	70	$\sim 10^6$
Chymotrypsinogen	350	2.8×10^5
Calmodulin: N-terminal	300-500	10^7
Calmodulin: C-terminal	10-20	

A body of biophysical measurements, mostly made before the advent of x-ray structures, indicated that CaM is constructed from two largely independent domains.[87] This conclusion emanated from studies of the two tryptic fragments, TR_1C and TR_2C. The major site of cleavage is between Lys-77 and Asp-78 of the central helix, and results in N-terminal and C-terminal fragments of nearly equal size. To a good approximation, the biophysical properties of the intact CaM molecule—NMR, UV and CD spectra, kinetic properties, thermochemical data, etc.—are the sum of the same properties of the fragments TR_1C and TR_2C. This means that we may assign the slow dissociation process, k_{off}^f, to the C-terminal domain, and the fast, k;ff' to the N-terminal domain of CaM. Combining binding constants and off-rates, we may calculate that the rates of Ca^{2+} binding to CaM are on the order of 10^7 M^{-1} s^{-1} at high ionic strength, and 10^8 M^{-1} s^{-1} or higher at low ionic strength. Recently the x-ray structure of the C-terminal fragment TR_2C was solved, and indeed showed a structure nearly identical with C-terminal domain of intact CaM.[91]

The structural changes occurring in CaM as Ca^{2+} ions are bound are associated with pronounced changes in ^1H NMR, UV, fluorescence, and CD spectra.[87] The observed changes in CD and fluorescence spectra in the presence of Mg^{2+} are only about 20 to

25 percent of those induced by Ca^{2+}. A comparison of the CD spectra of CaM and its **tryptic** fragments indicates that the structural changes induced by Ca^{2+} are substantially greater in the C-terminal than in the N-terminal half.[92] By and large, few structural details of the conformation changes have as yet been obtained. However, one aspect of the Ca^{2+}-induced conformation change is that *hydrophobic sites*, probably one on each domain of the molecule, become exposed. In the presence of excess Ca^{2+}, CaM will bind to other hydrophobic molecules, e.g., phenyl-Sepharose, a variety of drugs, many small peptides, and—last but not least—its target proteins. This brings us to the question of how CaM recognizes and interacts with the latter. We may suspect that the hydrophobic sites on each domain are somehow involved, but the role played by the central helix is still not clear. To explain small-angle x-ray scattering data, the interconnecting helix needs to be kinked, bringing the intact globular domains closer.[93]

A putative CaM-binding segment (27 amino acids long) of myosin light-chain kinase (MLCK), an enzyme activated by CaM, has been identified.[94] The interaction between the segment peptide ("M13") and CaM has been studied[95] by CD spectroscopy and ^1H NMR. From these studies it appears that a unique 1:1 complex is formed, and that secondary and tertiary structural changes occur not only in the peptide M13 but also in both halves of the CaM molecule. Further NMR studies 96,97 of the interaction between CaM and naturally occurring peptides (mellitin and mastoparan) that share some structural features of M13—clusters of basic residues, hydrophobic residues adjacent to the basic residues, and a predicted high α-helical content—show very much the same results. Based on these results, a model, shown in Figure 3.18, for the interaction between CaM and M13 has been proposed. In this model the central helix is kinked at position[81], allowing the two domains to wrap around the assumed α-helical M13. Preliminary structure calculations of calcium-loaded CaM, based on NMR data, indicate that the central helix in solution indeed is kinked and very flexible,[99] and comparisons[100] of chemical shifts in calmodulin with and without M13 complexed supports the model in Figure 3.18. Recent structural studies using NMR spectroscopy and x-ray diffraction have essentially confirmed the general features of this model, although the orientation of the peptide is found to be reversed.[173]

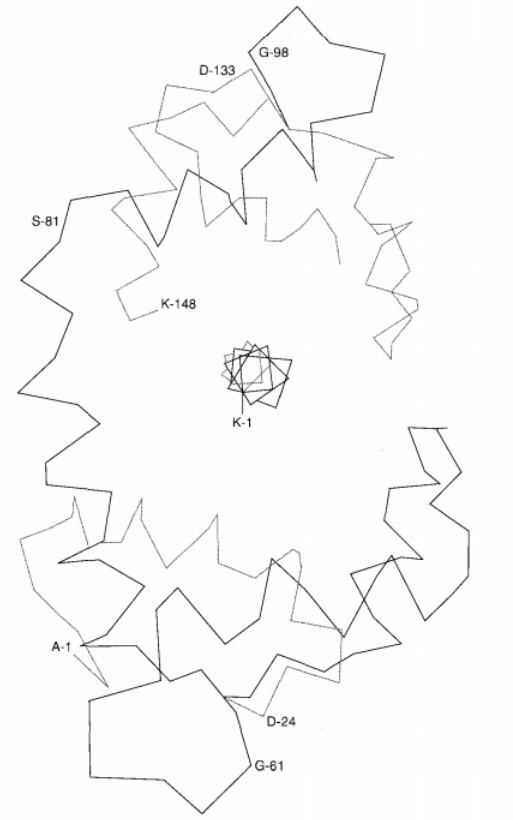

Figure kindly provided by R. Kretsinger; see also Reference 98.

In conclusion, two important features of the protein should be recognized.

i. The binding of Ca^{2+} to CaM (and to its complex with the target protein) is quite likely *cooperative*, meaning that the switch from inactive to active conformation may occur over a much more narrow Ca^{2+}-concentration interval than otherwise.
ii. The effective Ca^{2+} affinity will be different in the presence of the target proteins. To illustrate this second point, consider the standard free energies in the minimum scheme depicted in Figure 3.19. If the affinity of the Ca^{2+}-calmodulin complex

$(CaM(Ca)_4)$ for the target protein (P) is greater than that of Ca^{2+}-free calmodulin (CaM)—i.e., $|\Delta G_{III}| > |\Delta G_{II}|$—it follows that the Ca^{2+} affinity of the complex between P and CaM (P•CaM) must be higher than in CaM itself. This effect is also found experimentally in model systems.[101]

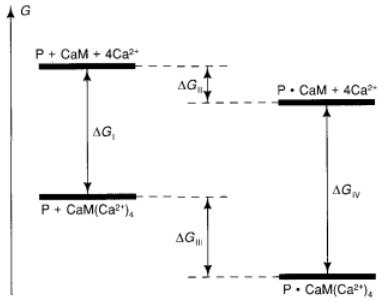

Figure 3.19 - Scheme depicting the standard free energies of different states in a system consisting of Ca^{2+}, calmodulin (CaM), and a target protein (P). P•CaM denotes a complex between calcium-free CaM and P, P•CaM$(Ca^{2+})_4$ denotes a complex with Ca^{2+}-loaded CaM. If the affinity of the Ca^{2+}-loaded CaM with the target protein P is higher than that of the Ca^{2+}-free form—i.e., $|\Delta G_{III}| > |\Delta G_{II}|$—it follows that the Ca^{2+} affinity of the complex p. CaM is higher than that of CaM itself.