Mazer, N. A., Benedek, G. B., & Carey, M. C. (1980) *Biochemistry 19*, 601.
McIntosh, T. J., Simon, S. A., & MacDonald, R. C. (1980) *Biochim. Biophys. Acta 597*, 445.
Menger, F. M. (1979) *Acc. Chem. Res. 12*, 111.
Menger, F. M., & Bonicamp, J. M. (1981) *J. Am. Chem. Soc. 103*, 2140.
Missel, P. J. (1981) Ph.D. Thesis, Massachusetts Institute of Technology.
Missel, P. J., Mazer, N. A., Benedek, G. B., Young, C. S., & Carey, M. C. (1980) *J. Phys. Chem. 84*, 1044.
Mukerjee, P., & Cardinal, J. R. (1978) *J. Phys. Chem. 82*, 1620.
Narayanan, R., Paul, R., & Balaram, P. (1980) *Biochim. Biophys. Acta 597*, 70.
Pownall, H. J., Morrisett, J. D., Sparrow, J. T., Smith, L. C., Shephered, J., Jackson, R. L., & Gotto, A. M., Jr. (1979) *Lipids 14*, 428.
Ribeiro, A. A., & Dennis, E. A. (1975) *Biochemistry 14*, 3746.
Roberts, M. F., & Dennis, E. A. (1978) in *Biomolecular Structure and Function* (Loeppky, R., & Agris, P., Eds.) pp 71–78, Academic Press, New York.
Roberts, M. F., Otnaess, A.-B., Kensil, C. A., & Dennis, E. A. (1978) *J. Biol. Chem. 253*, 1252.
Russell, J. C., Whitten, D. C., & Braun, A. M. (1981) *J. Am. Chem. Soc. 103*, 3219.
Scanu, A. M., & Landsberger, F. R. (1980) *Ann. N.Y. Acad. Sci. 348*, 1.
Shipley, G. G., Atkinson, D., & Scanu, A. M. (1972) *J. Supramol. Struct. 1*, 98.
Tausk, R. J. M., Karmiggelt, J., Oudshoorn, C., & Overbeek, J. Th. G. (1974a) *Biophys. Chem. 1*, 175.
Tausk, R. J. M., Van Esch, J., Karmiggelt, J., Voordouw, G., & Overbeek, J. Th. G. (1974b) *Biophys. Chem. 1*, 184.
Tausk, R. J. M., Oudshoorn, C., & Overbeek, J. Th. G. (1974c) *Biophys. Chem. 2*, 53.
Verger, R., & de Haas, G. H. (1976) *Annu. Rev. Biophys. Bioeng. 5*, 77.
Weast, R. C., Ed. (1980) *CRC Handbook of Chemistry and Physics*, 61st ed., p F57, CRC Press, Boca Raton, FL.
Wells, M. A. (1974) *Biochemistry 13*, 2248.
White, S. H., King, G. I., & Cain, J. E. (1981) *Nature (London) 290*, 161.
Worcester, D. L., & Franks, W. P. (1976) *J. Mol. Biol. 100*, 359.
Yeagle, P. L., Hutton, W. C., Huang, C.-H., & Martin, R. B. (1977) *Biochemistry 16*, 4344.

Studies of Calmodulin Structure: Laser Raman Spectroscopy of Biomolecules†

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ABSTRACT: The structure of bovine brain calmodulin was probed by using laser Raman spectroscopy to elucidate cation-induced conformational changes in the protein. Local changes, most likely reflecting metal binding but not rearrangement of the peptide backbone, were observed in the presence of calcium or magnesium. A conformational change involving the peptide backbone and secondary structure content of calmodulin was observed only in the presence of calcium. The calcium-induced conformational change in the peptide backbone involves increased α helix and β sheet. This was the only major calcium-specific change observed in the Raman spectrum, which suggests that the flexibility of the backbone conformation may play a critical role in the physiological activity of calmodulin.

Calmodulin is a small, acidic, intracellular calcium-binding protein that influences the activity of a number of enzymic and structural systems in the cell in a calcium-dependent manner [for reviews, see Klee et al. (1980), Cheung (1980), Brostrom & Wolff (1981), and Means et al. (1982)]. The protein, ubiquitous in eukaryotes, is one of the principal targets for intracellular calcium released following external stimuli. Calmodulin becomes activated when it binds calcium, undergoes conformational change, and complexes with its target protein.

Several metal ions, including Mg^{2+} and Mn^{2+}, have been shown to compete with calcium for binding sites on calmodulin but typically cannot induce biological activity in the protein (Wolff et al., 1977; Haiech et al., 1981). Conformational changes in calmodulin on binding various cations have been investigated in studies involving NMR[1] (Forsén et al., 1980; Seamon, 1980; Seamon & Moore, 1980), fluorescence (Kilhoffer et al., 1980, 1981; LaPorte et al., 1980, 1981; Tanaka & Hidaka, 1980; Kohse & Heilmeyer, 1981), CD (Dedman et al., 1977; Klee, 1977; Wolff et al., 1977), and ORD (Liu & Cheung, 1976). These studies generally agree that unique conformational states are associated with the binding of different metal ions.

Although some side chain groups and regions of the polypeptide backbone have been reported as undergoing cation-dependent structural changes, there is at present no precise identification of the physiologically relevant rearrangement. Laser Raman spectroscopy is a useful spectroscopic probe for investigation of both main chain and side chain groups in

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[1] Abbreviations: CD, circular dichroism; NMR, nuclear magnetic resonance; ORD, optical rotatory dispersion; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; cAMP, adenosine cyclic 3',5'-phosphate; Tris, tris(hydroxymethyl)aminomethane; UV, ultraviolet; pH*, uncorrected pH meter reading in deuterated solvent.

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proteins. This paper reports structural features associated with the peptide backbone and side chain residues in calmodulin that vary with the presence of calcium, magnesium, or monovalent cations, as detected by laser Raman spectroscopy.

Experimental Procedures

All chemicals were of the highest purity available from commercial sources. Distilled deionized water was provided by a Hydro Service Supplies, Inc., system and contained less than 1 ppm total ionic contaminants. Dialysis tubing was Spectrapor 6 with a molecular weight cutoff of 2000. Before use, the tubing was soaked in EGTA or EDTA solution and then in distilled deionized water and rinsed exhaustively. The same treatment was applied to all glassware used during the metal-removing steps. Stock solutions were stored in plastic containers. Wherever possible, Teflon vials and utensils (Pierce and Sci/Spec) were used.

Protein Preparations. Calmodulin was purified from bovine brain as described by Head et al. (1982). Calmodulin-dependent cyclic-nucleotide phosphodiesterase was purified from bovine brain as described by Head et al. (1979).

Phosphodiesterase Assay. Phosphodiesterase activity was measured at 37 °C with a final substrate concentration of 5 μM 3′,5′-cAMP by using the method of Boudreau & Drummond (1975).

Electrophoresis. Alkaline gel electrophoresis was carried out on slab gels (6 × 20 cm) of 8% w/v acrylamide containing 40% v/v glycerol and in a continuous buffer system of 25 mM Tris–80 mM glycine, pH 8.6, as described by Head & Perry (1974). Samples of calmodulin (1 mg/mL) were prepared containing 1–2 mM concentrations of free Ca^{2+}, Mg^{2+}, Na^+, or K^+. Each sample was initially dissolved in 1 mM EGTA to reduce free calcium levels ($<10^{-7}$ M) and subsequently adjusted to 2 mM total concentration of $CaCl_2$, $MgCl_2$, NaCl, or KCl. Since the binding of Mg^{2+}, Na^+, and K^+ to EGTA is several orders of magnitude weaker than that of Ca^{2+}, samples containing these cations still contained no more free calcium than $\sim 10^{-7}$ M. The sample containing added calcium had free calcium levels close to 1 mM. Bovine serum albumin (BSA) at 1 mg/mL was also included in each sample to act as an internal reference marker following staining. BSA has a mobility under these conditions approximately half that of calmodulin.

Preparation of Metal-Free Calmodulin. Calmodulin was freed from its bound metal ions by utilizing its inability to chelate cations at low pH (Haiech et al., 1981). Lyophilized calmodulin was dissolved in distilled deionized water to a protein concentration of approximately 8–10 mg/mL and dialyzed against 100 mM acetate at pH 3.5 (prepared by titrating distilled acetic acid with NaOH or distilled triethanolamine). Protein precipitation occurred upon contact with this solution. After dialysis against acetate buffer for several hours at 22 °C, the protein was dialyzed for up to 24 h at the same temperature, with frequent changes of dialysate, against distilled deionized water to which 0.05% chlorobutanol or 0.02% sodium azide had been added. The precipitated calmodulin was then dialyzed against $\sim$100 volumes of 50 mM Tris-HCl buffer, pH 7.0. The precipitate began to redissolve upon contact with the reservoir, and the solution appeared clear after several hours. The last step was dialysis against $\sim$1000 volumes of distilled deionized water, to remove buffer and bacteriostatic agents, followed by lyophilization. Lyophilized protein samples were stored below 0 °C and used within a month of preparation.

UV spectra of samples were recorded by a Perkin-Elmer λ 3 double-beam scanning UV–visible spectrophotometer. Protein concentration was determined as described by Crouch & Klee (1980). Atomic absorption measurements of calcium content were made on a Perkin-Elmer Model 703 spectrophotometer.

Preparation of Samples for Raman Spectroscopy. Protein samples were prepared by dissolving lyophilized metal-free calmodulin in the buffer solutions. Samples consisted of 150 mg/mL calmodulin in approximately 10 μL of buffer. Sodium or, in the case of potassium-loaded samples, potassium was present in at least 200 mM amounts in the buffer to prevent sample aggregation. Each sample was centrifuged at $\sim$13000*g* for 5 min and then introduced into a small (0.8–1.10 mm) closed-end Kimax capillary by a hypodermic syringe. Centrifugation at 400*g* for 1 min brought the sample droplet down to the closed end of the capillary. A small plug of melted wax was applied to the open end of the capillary to give a complete seal.

Deuterated buffer solutions were prepared by dissolving solid Tris and salt in 99.7% ^2H_2O, lyophilizing, and redissolving in 99.996% ^2H_2O. These solutions were titrated to pH* 7.7 with ^2HCl and NaO^2H. Protein solutions were prepared by dissolving lyophilized calmodulin in 99.7% ^2H_2O, lyophilizing, and redissolving in deuterated buffer solution. Complete exchange after this treatment was confirmed by NMR. The solutions were then prepared for the Raman experiments as described above.

Raman Spectra. The radiation source was a CR3 argon ion laser (Coherent Radiation). The spectrometer was a Spex Ramalog 4 double monochromator and detector (with a digital photometer). The 488-nm laser excitation line was used through a 488-nm filter. Power at all samples was 260–300 mW.

The spectra in Figures 3–5 were recorded with a spectral slit width of 4 cm^{-1}, scan speed of 0.05 cm^{-1}/s, gain of 1×10^4, photon counting mode with 1-s counting time, and 10-s smoothing. The spectra in Figure 2 were recorded with a slit width of 6 cm^{-1}, scan speed of 0.2 cm^{-1}/s, gain of 2×10^4, photon counting mode with 1-s counting time, and 5-s smoothing. All figures are tracings of original, rather than redrawn, spectra. Numbers written over peaks in Figures 2–5 refer to frequencies in cm^{-1}, and Raman intensity is represented on the ordinate. Frequencies are accurate to 1–2 cm^{-1} for sharp lines and to 3 cm^{-1} for broad bands.

Results

Preparation of Metal-Free Calmodulin. Atomic absorption measurements indicated the presence of less than 0.2 mol of Ca^{2+} bound per mol of calmodulin after the calcium removal procedure. The UV spectrum (Figure 1) of the treated calmodulin showed spectral features typical of calcium-deficient calmodulin (Klee, 1977). The calmodulin samples showed no quantitative degradation of biological activity after this treatment as indicated by calcium-dependent activation of calmodulin-dependent brain cyclic-nucleotide phosphodiesterase.

Sample Handling. For evaluation of the effects of irradiation on calmodulin samples, samples which had been in the laser beam for as long as 10 h were assayed for biological activity after the spectra were recorded. The samples fully activated cyclic-nucleotide phosphodiesterase. The temperature rise over the time period required for the recording of a typical Raman spectrum was between 1 and 2 °C (Gilbert et al., 1982).

Raman Spectra of Calmodulin. Amide I ($\sim$1650 cm^{-1}) and Amide III ($\sim$1250 cm^{-1}) Regions. The amide I region in the Raman spectrum of calmodulin in buffer solutions

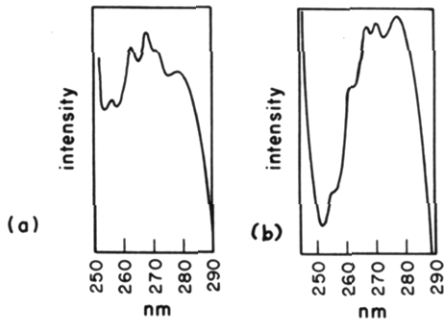

FIGURE 1: UV spectra of calmodulin (~10 μM) before (a) and after (b) calcium removal treatment.

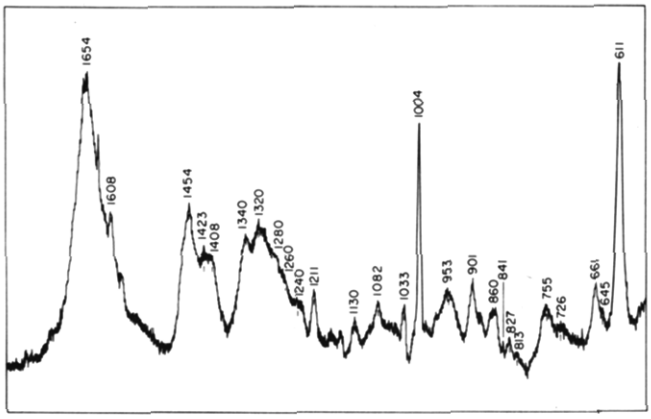

FIGURE 2: Raman spectrum of 150 mg/mL calmodulin in 0.2 M sodium cacodylate buffer, pH 7.0, with 0.1 M calcium chloride.

containing H_2O (Figure 2) is dominated by a strong peak at 1654 cm^{-1}, characteristic of α helix. Absence of intensity above 1670 cm^{-1} in H_2O–buffer rules out appreciable β sheet. In buffer solutions containing ^2H_2O, the amide I peak shifts to 1645 cm^{-1} (amide I′) which confirms the presence of large amounts of α helix.

In the amide III region (Figure 3), all calmodulin spectra exhibit strong intensity above 1260 cm^{-1}, at frequencies associated with α helix (1260–1300 cm^{-1}). Peaks appearing in the calmodulin spectra near 1250 cm^{-1} reflect the presence of intermediate structure.[2] Calmodulin samples in ^2H_2O–buffer (Figure 3, inset) show disappearance of peak intensity between 1235 and 1285 cm^{-1}, and a partial (~50%) decrease in peak intensity at 1300 cm^{-1}, relative to calmodulin samples in H_2O–buffer (Figure 3a–d). These data indicate that peaks appearing between 1240 and 1285 cm^{-1} are due to amide III vibrations, with some contribution from overlapping methylene deformation bands. Peaks found at ~1320 and ~1340 cm^{-1} are largely unaffected by deuteration.

The amide III contours in calmodulin spectra show a pattern of intensity that is observed with predominantly α-helical proteins (see Discussion). Only one peak, that occurring near 1250 cm^{-1}, is clearly resolved in the amide III curve in spectra of calcium-deficient calmodulin (Figure 3a–c). However, in spectra of calcium-loaded calmodulin (Figure 3d), new bands are resolved at 1269 and 1285 cm^{-1} in the α-helix region of

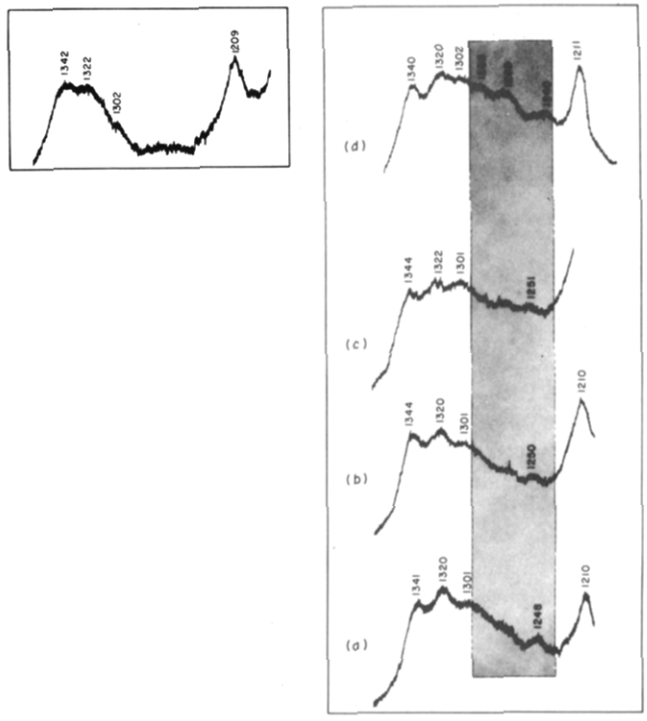

FIGURE 3: Raman spectra of calmodulin, amide III spectral region. Shaded area highlights region that responds to the presence of calcium with increased intensity at 1240, 1269, and 1285 cm^{-1}, with a decrease at 1250 cm^{-1}. (a) 150 mg/mL calmodulin in 0.1 M Tris buffer, pH 7.3, in 0.2 M NaCl; (b–d) as in (a), except for (b) 0.6 M NaCl instead of 0.2 M NaCl, (c) 0.2 M $MgCl_2$ added, and (d) 0.2 M $CaCl_2$ added. (Inset) 150 mg/mL calmodulin in 0.1 M Tris–^2H_2O and 0.2 M NaCl, pH* 7.7.

the amide III. In addition, a new peak appears at 1240 cm^{-1}, displacing the peak observed near 1250 cm^{-1} in the calcium-deficient samples.

Side Chain Chromophores. The region of the Raman spectrum of calmodulin between ~1400 and 1420 cm^{-1}, attributed to carboxylate groups on acidic side chains, exhibits intensity changes that are cation dependent. In general, as metal complexation by calmodulin increases, spectral intensity below 1410 cm^{-1} decreases relative to that observed near 1420 cm^{-1} (Figure 4).

Two peaks associated with tyrosine residues appear in the Raman spectrum near 825 and 855 cm^{-1}. Their intensity ratio has been correlated with the environment of the phenolic OH (Siamwiza et al., 1975). In the presence of monovalent cations,[3] two peaks of roughly equal intensity appear in this region of the calmodulin spectrum. When calcium is added, an additional peak at 864 cm^{-1} appears. This higher intensity doublet is also observed in the presence of magnesium but is less pronounced (Figure 5). While this change reflects cation-dependent conformational differences in calmodulin, the unequivocal assignment of the structural origin of this spectral feature is difficult.

Methionine peaks in the Raman spectrum show conformational sensitivity (Nogami et al., 1975). The strongest methionine bands appear at ~650, 700, and 724 cm^{-1}. In all calmodulin spectra, the 700-cm^{-1} peak is absent, and the 650- and 724-cm^{-1} peaks are observed.

The strong, sharp phenylalanine ring breathing peak at 1004 cm^{-1}, which lacks environmental sensitivity, was used as an

[2] Structure giving rise to amide III frequencies between those of α helix and β sheet are commonly referred to as random coil, disordered, etc., in the literature. We have used the term intermediate structure in the same context to denote peptide bonds with ψ angles intermediate to those found in pure α helices and β sheets (Ramachandran & Sasisekharan, 1968). The relationship between peptide backbone ϕ, ψ angles and corresponding amide III vibrations has been discussed in detail by Lord (1977).

[3] Raman samples containing potassium give identical spectra with those that contain only sodium; therefore, spectra of potassium-containing samples are not included in the figures.

internal frequency and concentration standard.

Electrophoretic Mobility. Calcium-induced structural changes in calmodulin, and in the homologous protein troponin C, have been found to give rise to changes in the size or shape of the molecule such that its mobility on alkaline gels is also altered (Amphlett et al., 1976; Head & Perry, 1974). In an attempt to relate the cation-induced structural changes in calmodulin, as detected by Raman spectroscopy, with such mobility changes, we have electrophoresed the protein in the presence of Na^+, K^+, Mg^{2+}, and Ca^{2+} ions, as described under Experimental Procedures. When the mobility of calmodulin in the presence of sodium ion was taken as unity, the relative mobilities in the presence of K^+, Mg^{2+}, and Ca^{2+} were found to be 0.98 ± 0.03, 0.98 ± 0.02, and 0.90 ± 0.03, respectively. These results suggest that under the electrophoretic conditions used, only calcium is able to produce those changes in the calmodulin molecule that give rise to a significant change in mobility (~10%) on this gel system. This is further evidence that the changes induced by calcium are qualitatively different from those brought about by magnesium and monovalent cations.

Discussion

Secondary Structure in Calmodulin. Amide I (~1650 cm^{-1}) and Amide III (~1250 cm^{-1}) Regions. Studies of the amino acid sequence of calmodulin relates the protein structurally to parvalbumin and other intracellular calcium-binding proteins containing "E–F hands" (Tufty & Kretsinger, 1975). Each E–F hand is composed of two helical regions, connected by a calcium-binding loop. The sequence data suggest the presence of four such "hands" in each calmodulin molecule. While this structural arrangement has not been confirmed crystallographically, CD studies of the protein have shown it to contain 30–55% α helix, depending upon experimental conditions (Dedman et al., 1977; Klee, 1977; Wolff et al., 1977). The Raman data confirm that calmodulin contains a large amount of α helix, which is consistent with the E–F hand prediction. The overall contour of the amide III region of calmodulin (Figure 3) strongly resembles those in the Raman spectra of other predominantly α-helical proteins, including bovine serum albumin (Chen & Lord, 1976), mojave (rattlesnake) toxin (Tu et al., 1976), and two viral coat proteins (Thomas & Murphy, 1975).

CD studies have also noted an increase of 5–15% α helix in calmodulin when calcium is added to the calcium-deficient protein. This is consistent with the increased intensity in the Raman spectrum at 1269 and 1285 cm^{-1}, in that the calcium-induced change is small but detectable. As with the CD results, the Raman data find little difference in the protein backbone conformations in the presence of cations other than calcium.

Apart from an increase in helix content, the Raman data indicate change in the intermediate secondary structure of calmodulin upon calcium binding. Calcium-deficient samples exhibit a peak near 1250 cm^{-1}, a frequency that is associated with neither α helix nor β sheet. When calcium is added to the protein, intensity at 1250 cm^{-1} (intermediate structure) decreases while intensity at 1240 cm^{-1} (β sheet) and above 1260 cm^{-1} (α helix) increases. Following the E–F hand model, these changes may be occurring in calcium-binding segments of those regions linking helices of different hands.

In Raman spectra of proteins, the extended β-sheet band typically appears between 1230 and 1240 cm^{-1}. In the presence of calcium, calmodulin spectra show a broad band near 1240 cm^{-1}. The actual amount of β sheet in calcium-bound calmodulin is difficult to assess because it appears to be a very minor structural component. Since β sheet tends not to exist as small, isolated segments, the 1240-cm^{-1} peak in calcium-loaded calmodulin more likely represents a backbone segment with a configuration and H-bonding pattern typical of β sheet that perhaps spans distant portions of the peptide but not as an extended chain. This arrangement has been noted in the crystal structures of parvalbumin (Kretsinger & Nockolds, 1973) and vitamin D dependent intestinal calcium-binding protein (Szebenyi et al., 1981). In the latter protein, distant stretches of two calcium-binding loops are H bonded to each other in a manner "resembling a small piece of β sheet".

Tertiary Structure in Calmodulin. Carboxylate Groups, 1400–1430-cm^{-1} Region. The COO^- symmetrical stretching vibration of ionized carboxylate groups in proteins typically gives rise to a Raman peak that appears near 1415 cm^{-1} (Lord & Yu, 1970). In calmodulin, intensity appears below 1420 cm^{-1} that is sensitive to perturbation by cation binding. The insensitivity to change upon deuteration (Figure 4, inset) supports the assignment of this peak as arising from carboxylate groups.

The Raman spectrum of troponin C displays calcium-dependent shifts in the frequency of peaks attributed to carboxylate groups (Carew et al., 1980). On the basis of their assignments, peak intensity between 1400 and 1410 cm^{-1} arises from uncomplexed carboxylate groups while those involved in metal ion chelation show intensity at slightly higher frequency. Trends observed in calmodulin spectra are consistent with their findings. Figure 4 shows that as the amount of cation complexation increases (a < b < c < d < e), the region between 1400 and 1420 cm^{-1} appears to broaden. As the ratio of uncomplexed to complexed carboxylate groups decreases, intensity shifts from ~1405 to ~1415 cm^{-1}, where it can no longer be resolved from the higher frequency methylene peak at ~1423 cm^{-1}.

Cation-induced conformational changes in calmodulin are not confined to the peptide backbone and carboxylate groups but also affect the configurations of other side chains. Aside from changes observed near the tyrosine doublet above 800 cm^{-1} (Figure 5), changes are also observed in the 1450–1460-cm^{-1} region due to methyl and methylene vibrations of protein side chains. The spectra show a cation-dependent frequency shift: the presence of magnesium or calcium is associated with a peak at 1454–1455 cm^{-1}, while monovalent cations are associated with a peak at 1450 cm^{-1}.

No cation-induced change is observed in the 725-cm^{-1} methionine peak in calmodulin; in all spectra, the overall configuration of the C–C–S–C group appears to be trans–trans (Nogami et al., 1975). While studies have implicated methionines in calmodulin activity (Walsh & Stevens, 1977), the Raman data show that conformational change involving methionine residues does not involve net rotation of those side chains.

Conclusion

The Raman results indicate that in calmodulin, there are at least two overall levels of conformational change induced by cations binding to the protein. One involves the peptide backbone and is specifically and solely induced by calcium. This change in structure is relatively small, since calcium-deficient calmodulin is predominantly α helix (with only small regions of intermediate structure that are incipient α helix or, to a lesser extent, β sheet). The electrophoresis results support the existence of a calcium-induced conformation that is different from those induced by other cations, although the nature of the difference is not clear from these experiments. This is in agreement with sedimentation coefficient determinations

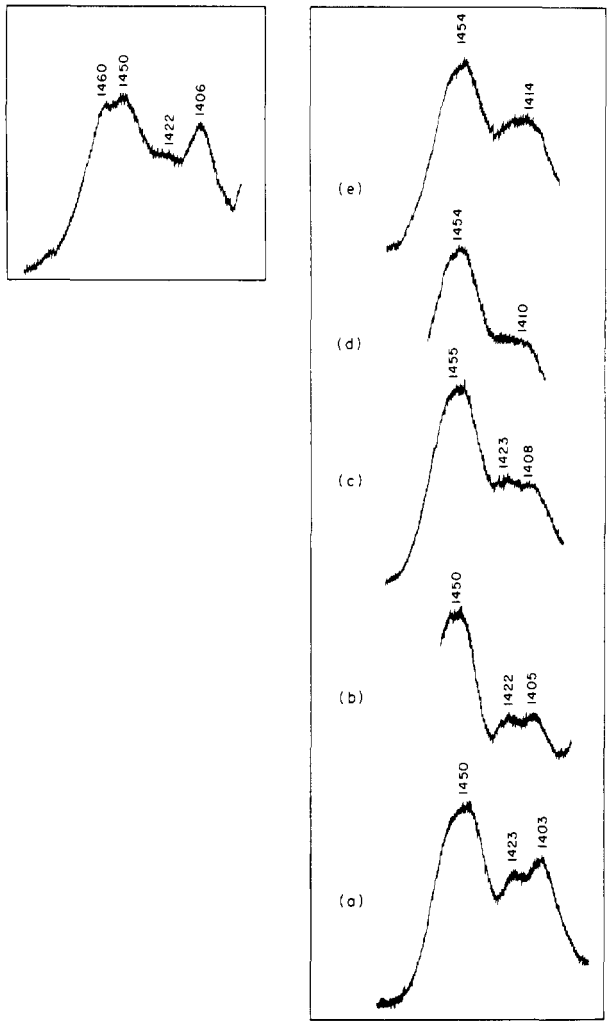

FIGURE 4: Raman spectra of calmodulin, 1400–1480-cm^{-1} region. (a) 150 mg/mL calmodulin in 0.1 M Tris buffer, pH 7.3, in 0.2 M NaCl; (b–e) as in (a), except for (b) 0.6 M NaCl instead of 0.2 M NaCl, (c) 0.2 M $MgCl_2$ added, (d) 0.1 M $CaCl_2$ added, and (e) 0.2 M $CaCl_2$ added. (Inset) 150 mg/mL calmodulin in 0.1 M Tris–^2H_2O and 0.2 M NaCl, pH* 7.7.

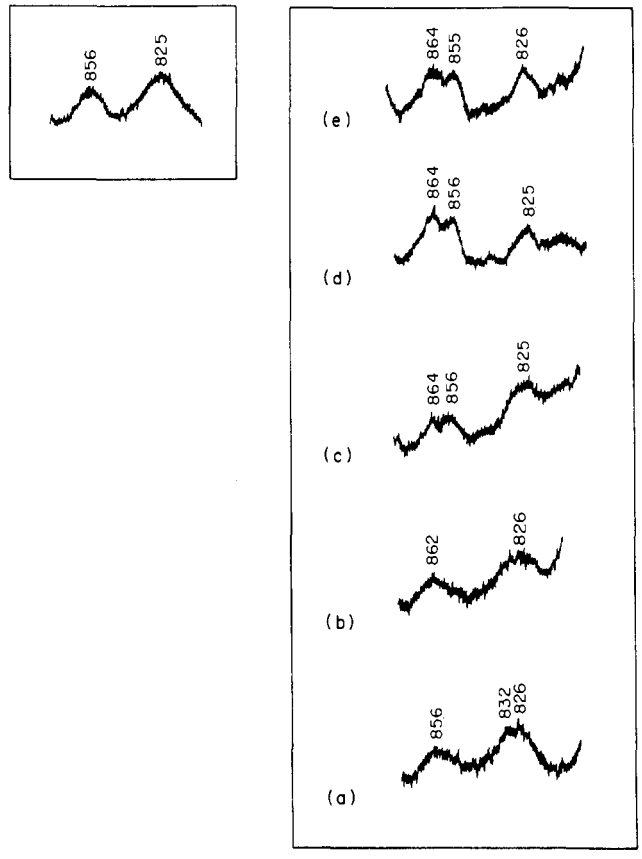

FIGURE 5: Raman spectra of calmodulin, 800–880-cm^{-1} region. (a) 150 mg/mL calmodulin in 0.1 M Tris buffer, pH 7.3, in 0.2 M NaCl; (b–e) as in (a), except for (b) 0.6 M NaCl instead of 0.2 M NaCl, (c) 0.2 M $MgCl_2$ added, (d) 0.1 M $CaCl_2$ added, and (e) 0.2 M $CaCl_2$ added. (Inset) 150 mg/mL calmodulin in 0.1 M Tris–^2H_2O and 0.2 M NaCl, pH* 7.7.

(Klee et al., 1980), chemical modification (Walsh & Stevens, 1977; Richman, 1978; Richman & Klee, 1978; Walsh et al., 1978), and protease susceptibility studies (Walsh et al., 1977). When calcium binds to calmodulin, it induces a new backbone conformation, albeit one which is very similar to that of the apoprotein.

However, the Raman data also indicate a level of conformational change that is independent of backbone configuration. In inducing this change, magnesium is similar to calcium, rather than to monovalent cations, in its effects on the Raman spectrum of calmodulin. This change may be confined to divalent cations occupying metal-binding sites in the protein, as spectral variation is observed in frequencies arising from carboxylate, methyl and methylene, and phenolic moieties. In the absence of the three-dimensional structure of calmodulin, the nature of this change cannot be visualized in detail. However, a localized change consisting of side chain reorientation appears most likely.

Studies of those changes in the calmodulin molecule that are calcium specific give information that may lead to an understanding of the mechanisms enabling calmodulin to activate its multiple target enzymes. Laser Raman spectroscopy provides one window through which the behavior of calmodulin may be observed. Results obtained by using this technique, taken in conjunction with data derived from other methods, will ultimately yield a detailed picture of molecular events involved in calmodulin activity.

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Registry No. Ca, 7440-70-2; Mg, 7439-95-4.

References

Amphlett, G. W., Vanaman, T. C., & Perry, S. V. (1976) *FEBS Lett. 72*, 163–168.

Boudreau, R. J., & Drummond, G. I. (1975) *Anal. Biochem. 63*, 388–399.

Brostrom, C. O., & Wolff, D. J. (1981) *Biochem. Pharmacol. 30*, 1395–1405.

Carew, E. B., Leavis, P. C., Stanley, H. E., & Gergely, J. (1980) *Biophys. J. 30*, 351–358.

Chen, M. C., & Lord, R. C. (1976) *J. Am. Chem. Soc. 98*, 990–992.

Cheung, W. Y. (1980) *Science (Washington, D.C.) 207*, 19–27.

Crouch, T. H., & Klee, C. B. (1980) *Biochemistry 19*, 3692–3698.

Dedman, J. R., Potter, J. D., Jackson, R. L., Johnson, J. D., & Means, A. R. (1977) *J. Biol. Chem. 252*, 8415–8422.

Forsén, S., Thulin, E., Drakenberg, T., Krebs, J., & Seamon, K. (1980) *FEBS Lett. 117*, 189–194.

Gilbert, W. A., Lord, R. C., Petsko, G. A., & Thamann, T. J. (1982) *J. Raman Spectrosc. 12*, 173–179.
Haiech, J., Klee, C. B., & Demaille, J. G. (1981) *Biochemistry 20*, 3890–3897.
Head, J. F., & Perry, S. V. (1974) *Biochem. J. 137*, 145–154.
Head, J. F., Mader, S., & Kaminer, B. (1979) *J. Cell Biol. 80*, 211–218.
Head, J. F., Masure, H. R., & Kaminer, B. (1982) *FEBS Lett. 137*, 71–74.
Hincke, M. T., Sykes, B. D., & Kay, C. M. (1981) *Biochemistry 20*, 4185–4193.
Kilhoffer, M.-C., Demaille, J. G., & Gerard, D. (1980) *FEBS Lett. 116*, 269–272.
Kilhoffer, M.-C., Demaille, J. G., & Gerard, D. (1981) *Biochemistry 20*, 4407–4414.
Klee, C. B. (1977) *Biochemistry 16*, 1017–1024.
Klee, C. B., Crouch, T. H., & Richman, P. G. (1980) *Annu. Rev. Biochem. 49*, 489–515.
Kohse, K. P., & Heilmeyer, M. G. (1981) *Eur. J. Biochem. 117*, 507–513.
Kretsinger, R. H., & Nockolds, C. E. (1973) *J. Biol. Chem. 248*, 3313–3326.
LaPorte, D. C., Wierman, B. M., & Storm, D. R. (1980) *Biochemistry 19*, 3814–3819.
LaPorte, D. C., Keller, C. H., Olwin, B. B., & Storm, D. R. (1981) *Biochemistry 20*, 3965–3972.
Liu, Y. P., & Cheung, W. Y. (1976) *J. Biol. Chem. 251*, 4193–4198.
Lord, R. C. (1977) *Appl. Spectrosc. 31*, 187–194.
Lord, R. C., & Yu, N.-T. (1970) *J. Mol. Biol. 50*, 509–524.
Means, A. R., Tash, J. S., & Chafouleas, J. G. (1982) *Physiol. Rev. 62*, 1–39.
Nogami, N., Sugeta, H., & Miyazawa, T. (1975) *Chem. Lett.*, 147–150.
Ramachandran, G. N., & Sasisekharan, V. (1968) *Adv. Protein Chem. 23*, 283–437.
Richman, P. G. (1978) *Biochemistry 17*, 3001–3005.
Richman, P. G., & Klee, C. B. (1978) *Biochemistry 17*, 928–935.
Seamon, K. B. (1980) *Biochemistry 19*, 207–215.
Seamon, K. B., & Moore, B. W. (1980) *J. Biol. Chem. 255*, 11644–11647.
Siamwiza, M. N., Lord, R. C., Chen, M. C., Takamatsu, T., Harada, I., Matsuura, H., & Shimanouchi, T. (1975) *Biochemistry 14*, 4870–4876.
Szebenyi, D. M. E., Obendorf, S. K., & Moffat, K. (1981) *Nature (London) 294*, 327–332.
Tanaka, T., & Hidaka, H. (1980) *J. Biol. Chem. 255*, 11078–11080.
Thomas, G. J., & Murphy, P. (1975) *Science (Washington, D.C.) 188*, 1205–1207.
Tu, A. T., Prescott, B., Chou, C. H., & Thomas, G. J. (1976) *Biochem. Biophys. Res. Commun. 68*, 1139–1145.
Tufty, R. M., & Kretsinger, R. H. (1975) *Science (Washington, D.C.) 187*, 167–169.
Walsh, M., & Stevens, F. C. (1977) *Biochemistry 16*, 2742–2749.
Walsh, M., Stevens, F. C., Kuznicki, J., & Drabikowski, W. (1977) *J. Biol. Chem. 252*, 7440–7443.
Walsh, M., Stevens, F. C., Oikawa, K., & Kay, C. M. (1978) *Biochemistry 17*, 3924–3930.
Wolff, D. J., Poirier, P. G., Brostrom, C. O., & Brostrom, M. A. (1977) *J. Biol. Chem. 252*, 4108–4117.