Calmodulin

DEVELOPMENT AND APPLICATION OF A SENSITIVE RADIOIMMUNOASSAY*

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Calmodulin has been radioiodinated by the Bolton-Hunter procedure. This procedure results in incorporation of 1.5 mol of $^{125}$I/mol of protein and yields a specific radioactivity of 2400 Ci/mmol. The radioiodinated calmodulin retains complete biological activity as determined by its ability to activate calmodulin-deficient cyclic nucleotide phosphodiesterase from rat brain. A sensitive radioimmunoassay for calmodulin has been developed using this $^{125}$I calmodulin as tracer. The assay exhibits a limit of detection of 15 pg and an assay sensitivity of 115 pg with intra- and interassay variabilities of <3% and <5%, respectively. Purified calmodulins from bovine brain, rat testis, Renilla reniformis (sea pansy) and Arachis hypogaea (peanut) demonstrate identical immunological cross-reactivities. These findings support the structural data revealing the highly conserved amino acid sequence of this protein. A homologous calcium-binding protein, troponin C, required a 665-fold greater protein concentration to cause 50% reduction in binding of $^{125}$I-calmodulin. The slope of the troponin C competition curve was different than that for calmodulin suggesting divergence between these two proteins. No immunological cross-reactivity was observed by the muscle calcium-binding protein, parvalbumin, at 50,000-fold excess protein. Adaptation of the radioimmunoassay to cell and tissue extracts reveals that calmodulin levels in various tissues and cells were always greater when assayed by the radioimmunoassay as compared to the phosphodiesterase assay. In addition, significant levels of calmodulin were detected in Dictyostelium discoideum and Chlamydomonas reinhardii by the radioimmunoassay whereas no calmodulin was demonstrable using the phosphodiesterase assay.

Calmodulin is a heat-stable, $M_r = 17,000$, multifunctional calcium-binding protein. This protein has been shown to mediate the calcium regulation of a large number of intracellular enzyme systems. These enzymes include a calcium-dependent form of Mg$^{2+}$-ATPase (12-15), myosin light chain kinase (16-19), adenyl cyclase (10,11), human erythrocyte membrane (Ca$^{2+}$, Mg$^{2+}$)-ATPase (12-15), myosin light chain kinase (20), and pea NAD$^+$ kinase (21). Calmodulin has also been reported to be the calcium-binding protein regulating calcium transport in the sarcoplasmic reticulum (22) and autophosphorylation of membrane proteins (23-25). In addition, immunofluorescence studies on a variety of cultured cells have demonstrated that calmodulin is localized on the actomyosin-containing stress fibers in interphase and is a dynamic component of the mitotic apparatus (26-28). In this regard, this protein has been shown to regulate the calcium-dependent assembly-disassembly of microtubules in vitro (29). Calmodulin has been considered to be ubiquitous in eukaryotes (30-32), and the primary structure appears to be highly conserved throughout the animal kingdom (33-35). These facts reflect the important role of calmodulin as an intracellular calcium-binding protein.

Calmodulin levels have been determined primarily by examining the ability of this protein to activate calmodulin-deficient cyclic nucleotide phosphodiesterase from mammalian tissue. The phosphodiesterase assay may not accurately measure the total levels of calmodulin since this assay measures only one of the many biological activities of the protein. Indeed, although all cells contain calmodulin, many cells do not contain a calmodulin-activatable form of phosphodiesterase (30,31). Furthermore, multiple calcium-dependent calmodulin-binding proteins have been reported to be present in many tissues which inhibit the phosphodiesterase assay (36-38). A radioimmunoassay would obviate these difficulties in that it would reflect the highly conserved nature of the protein and should be calcium-independent. Using procedures previously reported by our laboratory for affinity purification of monospecific antibody for calmodulin (27), we have prepared a precipitating antibody to calmodulin in sheep. In this communication, we report the development of a sensitive radioimmunoassay for calmodulin using the sheep antibody. The application of this assay to tissue extracts reveals differences between the levels of calmodulin determined by the immunological and enzymic assays.

EXPERIMENTAL PROCEDURES

Materials

Avidin and bovine serum albumin fraction IV (BSA) were purchased from Sigma. Pancreatin was purchased from Calbiochem-Behring. Rabbit reticulocyte lysate in vitro translation kit was purchased from New England Nuclear. $^{125}$I-labeled N-succinimidyl-3-(I-hydroxy-phenyl) propionate ($^{125}$I-labeled Bolton-Hunter reagent) was purchased from Amersham. Purified calmodulin from Arachis hypogaea and Renilla reniformis were the gifts of Dr. Milton Cormier (University of Connecticut, Department of Pathology). Chlamydomonas reinhardii was the gift of Dr. Ursula Goodenough (Washington University School of Medicine, St. Louis, Missouri), and Dictyostelium discoideum was the gift of Dr. D. L. Taylor (Harvard University, Department of Cell and Developmental Biology).

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$^+$ This protein has been referred to as calcium-binding phosphoprotein (1), protein activator (2,3), protein modulator (4,5), troponin C-like protein (5), and calcium-dependent regulator (6,7).
Purification of Proteins

Calmodulin was purified from rat testis and bovine brain according to the procedure of Dedman et al. (7). Rabbit muscle troponin C was purified by the procedure of Potter and Gergely (39), and carp parvalbumin purified by the procedure of Kretsinger and Nockolds (40) as modified by Marcum et al. (28).

Phosphodiesterase Assay for Calmodulin

Calmodulin was assayed by its ability to activate a standard preparation of calmodulin-deficient phosphodiesterase from rat brain as described by Beale et al. (41).

Preparation of Cell and Tissue Extracts

Tissues to be assayed for calmodulin were collected on ice and homogenized in 10 mM imidazole, 1 mM EGTA, 1 mM 2-mercaptoethanol, 10 mM MgCl₂, 0.15 M NaCl, pH 7.5 (51 v/w) with a polytron homogenizer (five 30-s bursts using a PT-10 generator at a setting of 4.5). C. reinkhardtii and D. discoideum were prepared for assay by sonication in the homogenization buffer (51 v/w) with a Brownwill Biosonic IV Sonicator. Homogenates were brought to a rapid boil in a microwave oven, immediately removed, and immersed in a methanol dry ice bath to effect rapid cooling. Rabbit polymorphonuclear leukocytes (PMN) were prepared in a similar way but were heat-treated prior to homogenization. The homogenate samples were then centrifuged at 10,000 x g for 30 min and the supernatant solutions assayed.

Preparation of Antiserum and Purification of Antibody

Electrophoretically homogeneous rat testis calmodulin was prepared as previously described (7). The protein solution (0.9% NaCl solution) was emulsified in an equal volume of complete Freund's adjuvant and injected subcutaneously (lower back) into an adult sheep. Ten milligrams of calmodulin was injected on Day 0, followed by 2 mg insults on Days 42, 56, 70, and every 60 days thereafter. Bleedings were obtained at 2-week intervals. Monospecific anti-calmodulin was prepared from the sheep antiserum by the procedure of Dedman et al. (27) using affinity chromatography (rat testis calmodulin covalently bound to Sepharose 4B).

Radioiodination of Calmodulin

Bolton-Hunter Procedure—Rat testis calmodulin was radioiodinated by the Bolton-Hunter procedure (42). One mCi of [¹²⁵I]-labeled N-succinimidyl-3-(4-hydroxyphenyl)propionate ([¹²⁵I]-labeled Bolton-Hunter reagent (1600 Ci/mmol supplied in benzene by American) was evaporated to dryness under a stream of nitrogen. Five milligrams of rat testis calmodulin (90 pg) was radioiodinated in this manner routinely incorporated 1.5 mol of [¹²⁵I]-labeled Bolton-Hunter reagent. [¹²⁵I]-Calmodulin was separated from free [¹²⁵I]-labeled Bolton-Hunter reagent by chromatography on a Sephadex G-25 column (0.7 x 20 cm) previously equilibrated with 0.05 M CaCl₂, pH 7.5. Calmodulin radioiodinated in this manner routinely incorporated 1.5 mol of [¹²⁵I] of protein which resulted in a specific radioactivity of 2400 Ci/mmol.

Lactoperoxidase Procedure—One hundred twenty-five micrograms of rat testis calmodulin was radioiodinated enzymatically according to the procedure of David and Heisfeld (43). The [¹²⁵I]-calmodulin was separated from free [¹²⁵I] by Sephadex G-25 chromatography. Calmodulin radioiodinated in this manner had a specific radioactivity of 46.6 Ci/mmol.

Chloramine-T Procedure—Rat testis calmodulin (90 µg) was radioiodinated by the chloramine-T procedure as described by Leidenberger and Reichert (44) and processed as described above. Chloramine-T-radioiodinated calmodulin had a specific radioactivity of 18.64 Ci/mmol.

RESULTS

[¹²⁵I]-Calmodulins labeled by the three radioiodination methods were tested for their ability to activate calmodulin-deficient phosphodiesterase from rat brain, as demonstrated in Fig. 1. [¹²⁵I]-Calmodulin labeled with the Bolton-Hunter reagent retains complete biological activity. The activation curve is identical with that obtained by native protein and results in half-maximal activation at 7 ng. Lactoperoxidase-treated [¹²⁵I]-calmodulin exhibits considerably less biological activity with respect to native protein and demonstrates an activation curve significantly different from that of native protein. In this case, half-maximal activation is achieved with 21.5 ng of protein. The chloramine-T radioiodination procedure totally destroys the ability of calmodulin to activate phosphodiesterase. These data demonstrate that under the conditions used, the Bolton-Hunter procedure is the preferred method for radioiodinating calmodulin.

The Bolton-Hunter-radioiodinated [¹²⁵I]-calmodulin was analyzed by polyacrylamide gel electrophoresis in the presence of 0.1% SDS to verify authenticity (Fig. 2). The sliced gel revealed a single peak of [¹²⁵I] which co-migrates with authentic rat testis calmodulin.
rat testis calmodulin. Analysis under non-denaturing conditions also revealed a single peak of $^{125}$I coincident with authentic rat testis calmodulin (data not shown). $^{125}$I-Calmodulin radioiodinated by the Bolton-Hunter procedure was used for all subsequent experiments.

Radioimmunoassay—In order to provide a reagent of uniform concentration and titer, calmodulin affinity chromatography was used to purify anti-calmodulin from sheep serum. The ability of this calmodulin affinity-purified antibody to precipitate $^{125}$I-calmodulin was then tested. Two identical series of dilutions of calmodulin affinity-purified sheep anti-calmodulin were made (starting with 20 µg of anti-calmodulin/assay tube). A constant IgG concentration of 80 µg/assay tube was maintained using the IgG which was not retained on the calmodulin affinity column (nonabsorbed IgG) as carrier. The dilution profile of anti-calmodulin was obtained by adding 100 µl of the appropriate dilution of anti-calmodulin to 400 µl of radioimmunoassay buffer (0.125 M borate, 20 µg/ml of bovine serum albumin, 1 mM EGTA, 75 mM NaCl, pH 8.4) containing 10,000 cpm of $^{125}$I-calmodulin. Nonspecific binding of $^{125}$I-calmodulin was determined by substituting 100 µl of nonabsorbed IgG (80 µg/tube) for anti-calmodulin. Samples were first incubated for 2 h at 25°C and then overnight at 4°C. To one series of tubes, 10 µl of a 10% Pansorbin suspension was added. To the second series of tubes, 10 µl of Pansorbin buffer (0.125 M borate, 75 mM NaCl, 1 mM EGTA, pH 8.4) was added. All samples were incubated for 30 min at 25°C followed by centrifugation at 10,000 × g for 15 min. The pellets were washed three times with 1-ml aliquots of the radioimmunoassay buffer. Radioactivity of the resultant pellets was determined in a Searle y counter. Using the anti-calmodulin precipitation reaction for separation of antibody-bound $^{125}$I from free, 52% of the total $^{125}$I-calmodulin was precipitated at an antibody concentration of 20 µg/assay tube (Fig. 3). When Pansorbin (protein A) was employed in the separation phase, 91% of the total $^{125}$I-calmodulin was precipitated at the same antibody concentration. In both cases, there was a decrease in the precipitable $^{125}$I counts as the antibody was diluted. At each point, total precipitable $^{125}$I counts were greater when Pansorbin was present. No precipitation of $^{125}$I-calmodulin was observed when nonabsorbed IgG (80 µg/assay tube) was substituted for anti-calmodulin. In all subsequent assays 1.5 µg of anti-calmodulin and 10 µl of Pansorbin were used. These conditions resulted in 40% $^{125}$I bound.

Standard dilution curves for pure proteins were generated by mixing 100 µl of the appropriate dilution of the competing protein with 300 µl of radioimmunoassay buffer containing 10,000 cpm of $^{125}$I-calmodulin. One hundred microliters of anti-calmodulin was then added and the assay performed as described above. The immunological cross-reactivities of several purified proteins relative to rat testis $^{125}$I-calmodulin were investigated (Fig. 4). Calmodulin from rat testis, bovine brain, R. reniformis (sea pansy), and A. hypogaea (peanut) compete in an identical manner with rat testis $^{125}$I-calmodulin and describe the same curve. Troponin C from rabbit skeletal muscle demonstrates some immunological cross-reactivity. However, the slope of its competition curve is significantly less than that for calmodulin (~1.88 and ~1.88 for troponin C and calmodulin, respectively) and a 665-fold greater protein concentration is required for 50% competition. No immunological cross-reactivity is observed when up to 50 µg of the muscle calcium-binding protein, parvalbumin, or the egg white protein, avidin, are tested (50,000-fold protein excess).

The data presented in Fig. 5 and all subsequent data were calculated using the log-logit computer program based on the assay statistics derived by Midgley et al. (46) and Duddleson et al. (47). The radioimmunoassay has an interassay variability <5% and an intra-assay variability <3%. The statistical assay sensitivity (+2 S.D.) is 115 pg whereas the limit of detection is 15 pg. In all cases, background is <100 cpm.

Quantitation of Calmodulin in the Cell and Tissue Extracts by the Radioimmunoassay—The usual sample to be tested for calmodulin content by the radioimmunoassay would be a cell extract. To demonstrate the specificity of the antibody, it was necessary to show that immunoprecipitation of a solution containing a mixture of radioactive proteins yielded a single product. Therefore, total poly(A)-containing RNA was translated in a rabbit reticulocyte lysate cell-free translation system as described by Means et al. (48). Fig. 5 describes the results of a typical experiment. Panel B demonstrates the presence of many labeled proteins in the total translation product. Immunoprecipitation of this mixture resulted in a single radioactive peak (Panel C) which co-migrated with...
authentic $^{125}$I-calamodulin (Panel A). Specificity was verified by including 10 $\mu$g of authentic rat testis calmodulin in the immunoprecipitation reaction. Panel D shows the absence of a radioactive immunoprecipitable product.

The applicability of the radioimmunoassay in the detection and characterization of calmodulin from biological samples is demonstrated in Fig. 6. The electrophot of the electric eel (Electrophorus electricus) is a tissue that has been shown to contain calmodulin (49), and the slope of its competition curve is identical with the purified rat testis calmodulin. Extracts from rabbit polymorphonuclear leukocytes (PMN), the slime mold, D. discoideum, and the algae, C. reinhardii, also generate competition curves similar to rat testis calmodulin. These data demonstrate the utility of the radioimmunoassay in the detection of calmodulin from tissue extracts from divergent sources.

Comparison between Phosphodiesterase Assay and Radioimmunoassay—In order to ascertain whether calmodulin levels determined by the biological and immunological activity of the protein are comparable, equal aliquots of various tissue and cell extracts were assayed for calmodulin by both the phosphodiesterase assay and the radioimmunoassay (Table I). Four tissues from rat; heart, liver, brain, and testis demonstrate greater calmodulin levels when assayed by the radioimmunoassay. The differences in calmodulin levels as determined by the two assays are not constant among the four rat tissues but range from 292% in heart to only 11% in testis. An even greater difference was found using extracts from polymorphonuclear leukocytes in which case a 500-fold difference was detected. Moreover, while the radioimmunoassay could detect calmodulin in the slime mold, D. discoideum, and the algae, C. reinhardii, no activity could be detected by the phosphodiesterase assay.

DISCUSSION

As an initial step in the development of a sensitive radioimmunoassay for calmodulin, it was necessary to achieve a high specific radioactivity of the antigen while preserving both biological and immunological potency. Calmodulin was radioiodinated by the three most commonly used procedures, chloramine-T, lactoperoxidase, and Bolton-Hunter reagent. The oxidizing procedures chloramine-T and lactoperoxidase radioiodinate proteins by incorporation of $^{125}$I directly into tyrosine residues. Calmodulin contains 9 tyrosine residues which increase in fluorescence when the protein binds calcium. This physical property suggests that the tyrosine residues migrate to a more hydrophobic region of the protein and that this is important for the tertiary structure of the active molecule. Using these methods, the resultant radioiodinated calmodulin was of low specific radioactivity and had reduced or absent biological activity (Fig. 1). LaPorte and Storm (50) and Richman and Klee (51) have previously reported on the radioiodination of calmodulin by the lactoperoxidase procedure with retention of some biological activity. However, the specific radioactivity reported by Richman and Klee was 57 cpm/ng (51). The Bolton-Hunter procedure conjugates the $^{125}$I-labeled Bolton-Hunter reagent to the $\varepsilon$-amino side chains of lysine residues or the NH$_2$-terminal amino group (which is acetylated in calmodulin) via an amide bond. Calmodulin contains 7 lysine residues and this procedure produced $^{125}$I-

![Fig. 5. Polycrylamide gel electrophoretic analysis of anti-calmodulin immunoprecipitate. Total poly(A)-containing RNA was translated in a rabbit reticulocyte lysate cell-free translation system by the procedure of Means et al. (48) and the resulting $^{35}$S translation product immunoprecipitated with anti-calmodulin as described under "Experimental Procedures." Samples were analyzed on 17% polyacrylamide gels by the procedure of Laemmli (45). A, authentic rat testis $^{125}$I-calmodulin; B, total protein synthesized from the cell-free translation system; C, immunoprecipitate by anti-calmodulin (2 $\mu$g); D, immunoprecipitate by 2 $\mu$g of anti-calmodulin plus 10 $\mu$g of unlabeled rat testis calmodulin.

![Fig. 6. Competitive binding of various tissue and cell extracts with anti-calmodulin. Varying dilutions of the heat-treated supernatant solutions from the electroplax of E. electricus and the cells D. discoideum, C. reinhardii, and rabbit polymorphonuclear leukocytes (PMN) were assayed as described under "Experimental Procedures." The dilution of each sample which exhibited 50% competition was used to standardize each dilution curve to the rat testis calmodulin curve.

<table>
<thead>
<tr>
<th>Source</th>
<th>Radioimmunoassay</th>
<th>Phosphodiesterase</th>
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<tr>
<td>Rat heart</td>
<td>3.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Rat liver</td>
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<td>5.4</td>
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<tr>
<td>Rat brain</td>
<td>21.0</td>
<td>14.7</td>
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<tr>
<td>Rat testis</td>
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</tr>
<tr>
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<td>0</td>
</tr>
<tr>
<td>C. reinhardii</td>
<td>4.9</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE I

Comparison of calmodulin levels from various sources as determined by the radioimmunoassay and phosphodiesterase assay

Equal aliquots from the same tube of each heat-treated-sample were assayed for calmodulin by both assays as described under "Experimental Procedures." Calmodulin levels are expressed as nanograms per $\mu$l of sample supernatant.
Calmodulin Radioimmunoassay

calmodulin of high specific radioactivity (2400 Ci/mmol, 156,000 cpm/ng). Although the Bolton-Hunter-synthesized $^{125}$I-calmodulin is of high specific radioactivity, it still retains full biological and immunological activity. Furthermore, it is relatively stable and can be stored for up to 6 weeks at 4°C without any significant damage. These data are in agreement with previous studies showing retention of immunological and biological activity following radioiodination using the Bolton-Hunter method (42, 52, 53). Although the $^{125}$I-labeled Bolton-Hunter reagent was originally synthesized in our lab by the procedure described by Bolton and Hunter (42), it is also commercially available from Amersham. We have found that the commercial product is far more efficient and consistent in radiiodinating calmodulin as well as several other proteins.

Significant differences were observed between calmodulin levels determined by the phosphodiesterase assay and radioimmunoassay. In all samples tested, levels of calmodulin determined by the radioimmunoassay were greater. Indeed, only the radioimmunoassay is capable of detecting calmodulin from the slime mold, D. discoideum, or the algae, C. reinhardii, under the conditions employed. Since both assays use the same supernatant preparation, these differences cannot be attributed to different recoveries. In fact, this experiment was only designed to compare differences generated by the two assays and was not designed to quantitate total levels. In order to reduce any variability due to standardization, both assays were calibrated by the same rat testis calmodulin standard. The concentration of this standard calmodulin preparation was determined by amino acid analysis.

A possible reason for the discrepancies between the two assays is suggested by the conditions required for both assays. The determination of calmodulin levels by the phosphodiesterase assay is dependent on the activation of phosphodiesterase by the protein in a calcium-dependent manner. Intrinsic to this assay is the requirement for biologically active calmodulin and calcium-dependent binding. It is possible that some cells and tissues may contain calmodulin altered in such a fashion as to render it incapable of activating mammalian phosphodiesterase. This is supported by studies in D. discoideum where although exogenous calmodulin can substitute for the calcium-binding component required for gel-sol transformation, no calmodulin activity can be detected in extracts from the slime mold using the phosphodiesterase assay (Table I). Recently, Klee and Krinks (36) and Sharma et al. (37, 38) have reported the purification of two proteins that inhibit activation of phosphodiesterase by calmodulin. These proteins inhibit the enzyme activation by binding competitively to calmodulin in a calcium-dependent manner. While the protein described by Klee and Krinks (36) is heat-labile, the one described by Sharma et al. (37, 38) is heat-stable. Although samples tested for calmodulin are heat-treated in order to abolish endogenous phosphodiesterase activity, any such soluble heat-stable proteins that interact with calmodulin in a calcium-dependent manner would be present. The calmodulin-anti-calmodulin interaction, however, is calcium-independent (data not shown), and therefore, the radioimmunoassay is performed routinely in the presence of EGTA in order to preclude calcium-dependent interactions by calmodulin. The fact that the apparent differences between the two assays are not constant from tissue to tissue suggests that they may be due to different pools of biologically active calmodulin, or heat-stable calmodulin-binding proteins specific for each tissue, or both. Such differences would be reflected in the phosphodiesterase assay but not the radioimmunoassay. Data presented in this study would, therefore, suggest that calmodulin is present at greater concentrations and in more tissues than previously reported.

Since the determination of levels of a protein by a radioimmunoassay is predicated on comparing the degree of competitive binding by the sample to a standard competition curve, samples to be assayed must demonstrate similar immunological cross-reactivities to those standards. The slope of a competition curve generated by a protein is characteristic of that specific antigen-antibody interaction. Therefore, a competition curve identical with the standard curve would suggest identity of that protein with the standard protein. Similarly, the degree of divergence between the two proteins would be expressed as an alteration in immunological cross-reactivity with a subsequent decrease in the slope of the competition curve. The data presented in this paper demonstrate the similarity of the calmodulin competition curve using pure proteins and tissue extracts from diverse sources. The relative immunological cross-reactivities of two homologous muscle calcium-binding proteins, troponin C (rabbit skeletal) and parvalbumin (carp), were also tested. Troponin C, which has 50% amino acid sequence homology (39, 40) and significant biological cross-reactivity with calmodulin (54), required a 665-fold greater concentration for 50% competition. The slope of the competition curve was significantly less than that for calmodulin, suggesting that this immunological cross-reactivity is not due to calmodulin contamination in the protein preparation, but due to the divergence exhibited between these two proteins. Parvalbumin, on the other hand, which has little sequence homology or biological cross-reactivity (55), demonstrated no immunological cross-reactivity. Similarly, avidin, a protein of similar size to calmodulin but which neither binds calcium nor has any sequence homology and biological cross-reactivity with calmodulin, exhibited no detectable immunological activity.

The immunological identity of calmodulin from representative species of primitive algae, slime mold, and coelenterate to the more advanced plants and mammals is compatible with structural studies on the protein. These latter studies demonstrate that the amino acid sequence of calmodulin is invariant in structure in closely related species (33, 34) and the sea pen, R. reniformis (35), differing by no more than six conservative amino acid substitutions. Moreover, each of these proteins contain four internally homologous calcium-binding domains, and only one of the substitutions (in R. reniformis) occurs in these highly conserved regions. Taken together these data suggest that calmodulin is one of the most highly conserved proteins studied. Indeed, broad distribution and highly conserved primary sequence are criteria which should be met by a protein involved in the regulation of a variety of intracellular processes. Calmodulin regulates cell motility including ciliary motion, microfilament contraction, relaxation (19, 56), and the movement of the chromosomes to the poles during mitosis (26). These are fundamental processes that occur in all species from the most primitive unicellular organism to the most advanced mammal. In addition, calcium transport has been reported to be affected by calmodulin both within compartments of cells (22) and also via the plasma membrane calcium pump (12-15). Glycogen metabolism can be regulated by calmodulin via the activation of phosphorylase b kinase (20). Indeed, this may explain the mechanism by which α-adrenergic agonists regulate glycogen metabolism in a manner not requiring elevated levels of cyclic AMP (57-59). Finally, cyclic nucleotide metabolism can be affected by calmodulin in a variety of ways. Reports exist that show regulation of adenyl cyclase (10, 11), phosphodiesterase (8, 9), and...
calcareous dependent protein kinase (16-21, 23-25) by this cal- cium-binding protein.

Calmodulin is ubiquitous in eukaryotic cells and structurally conserved. This protein binds calcium with high affinity and specificity. In fact, it is the major calcium-binding protein in non muscle cells, and so far, no alternative function has been described except to bind calcium. Finally, calmodulin has multiple binding or acceptor proteins. It is likely that the calmodulin inhibitor proteins previously described (36-38) represent calmodulin-regulated enzyme activities which have yet to be defined. These multiple acceptor proteins may well be involved in the calcium regulation of other fundamental processes common to all organisms. Collectively, these data would suggest that calmodulin may be an intracellular calcium receptor. The calcium-calmodulin complex may represent the biologically active state of intracellular calcium. The radioim- munoassay provides a valuable tool that can be used to test this hypothesis from data obtained from studies of evolutionarily diverse species.

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Note Added in Proof—While this manuscript was in press, Wallace and Cheung reported the development of a radioimmunoassay for calmodulin (1979) J. Biol. Chem. 254, 6646-6671. This assay is 300-fold less sensitive for native calmodulin than the assay described in the present communication. A possible reason for the difference could be that the calmodulin used for antibody production by Wallace and Cheung was dinitrophenylated.

REFERENCES