Blocking the Ca\textsuperscript{2+}-induced Conformational Transitions in Calmodulin with Disulfide Bonds\textsuperscript{*}

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Calcium-dependent regulation of intracellular processes is mediated by proteins that on binding Ca\textsuperscript{2+} assume a new conformation, which enables them to bind to their specific target proteins and to modulate their function. Calmodulin (CaM) and troponin C, the two best characterized Ca\textsuperscript{2+}-regulatory proteins, are members of the family of Ca\textsuperscript{2+}-binding proteins utilizing the helix-loop-helix structural motif (EF-hand). Herzberg, Moult, and James (Herzberg, O., Moult, J., and James, M. N. G. (1986) J. Biol. Chem. 261, 2638–2644) proposed that the Ca\textsuperscript{2+}-induced conformational transition in troponin C involves opening of the interface between the α-helical segments in the N-terminal domain of this protein. Here we have tested the hypothesis that a similar transition is the key Ca\textsuperscript{2+}-induced regulatory event in calmodulin. Using site-directed mutagenesis we have substituted cysteine residues for Gln\textsuperscript{41} and Lys\textsuperscript{75} (CaM41/75) or Ile\textsuperscript{85} and Leu\textsuperscript{112} (CaM85/112) in the N-terminal and C-terminal domains, respectively, of human liver calmodulin. Based on molecular modeling, cysteines at these positions were expected to form intramolecular disulfide bonds in the Ca\textsuperscript{2+}-free conformation of the protein, thus blocking the putative Ca\textsuperscript{2+}-induced transition. We found that intramolecular disulfide bonds are readily formed in both mutants causing a decrease in affinity for Ca\textsuperscript{2+} and the loss of ability to activate target enzymes, phosphodiesterase and calcineurin. The regulatory activity is fully recovered in CaM41/75 and partially recovered in CaM85/112 upon reduction of the disulfide bonds with dithiotreitol and blocking the Cys residues by carboxamidomethylation or cyanylation. These results indicate that the Ca\textsuperscript{2+}-induced opening of the interfaces between helical segments in both domains of CaM is critical for its regulatory properties consistent with the Herzberg-Moult-James model.

Troponin C (TnC)\textsuperscript{1} and calmodulin (CaM) are the two best characterized Ca\textsuperscript{2+}-binding regulatory proteins and members of the EF-hand family of Ca\textsuperscript{2+}-binding proteins. The tissue distribution and the function of these proteins are different; TnC is the striated muscle-specific protein, whereas CaM is an ubiquitous protein involved in regulation of a number of intracellular enzymatic systems. In spite of different functions the two proteins have remarkably similar structures (1–3). There are eight helical segments (A–H) associated with the four helix-loop-helix Ca\textsuperscript{2+}-binding sites equally distributed between two globular domains. The most significant difference between crystallographic structures of the two proteins is the lack of Ca\textsuperscript{2+} sites I and II in TnC that results in a more compact packing of the N-terminal domain of this protein. The helices flanking the Ca\textsuperscript{2+}-binding loops in this domain make tight contacts through the side chains and run in an antiparallel fashion. In contrast, in the two Ca\textsuperscript{2+}-binding sites in the C-terminal domain of TnC and in all four sites in CaM the interhelical dihedral angle of a helix-loop-helix unit is close to 90°. This difference forms the basis for the mechanism of the Ca\textsuperscript{2+}-induced conformational transition proposed by Herzberg et al. (4). They proposed that upon Ca\textsuperscript{2+} binding to site I and II in TnC, the B/C pair of helices moves away from the A/D pair thereby exposing a patch of hydrophobic residues, the interaction site for TnI. This model, referred to as the Herzberg-Moult-James (HMJ) model, has a strong experimental support based on several lines of evidence (for review see Grabarek et al. (5)). The atomic resolution structure of the Ca\textsuperscript{2+}-filled N-terminal domain fragment of TnC recently solved by NMR (6) and x-ray crystallography (7) have proved the HMJ model to be correct.

Here we address the question of whether the HMJ model is unique for the N-terminal domain of TnC or whether it is also applicable to calmodulin. We have designed two mutants of human liver CaM in which the putative Ca\textsuperscript{2+}-induced opening of the interhelical interfaces in each of the two domains could be blocked by a disulfide bond. Cysteine residues have been introduced at position 41 and 75 in the N-terminal domain (CaM41/75) and at position 85 and 112 in the C-terminal domain (CaM85/112). We found that the cysteine residues in both mutants form stable disulfide bonds. The oxidized forms of these mutants are unable to activate phosphodiesterase and calcineurin. Upon reduction of the disulfide and blocking SH groups, the regulatory properties are fully restored in CaM41/75 and to a large extent in CaM85/112. These data strongly support the view that a TnC-like opening of the interhelical interface in each of the two domains of CaM represents the key Ca\textsuperscript{2+}-induced conformational transition in CaM.

MATERIALS AND METHODS

Subcloning Human Liver CaM cDNA to M13 mp19—The plasmid pKT218 containing the human liver calmodulin cDNA done (8) was a generous gift of Dr. Richard Perham (Cambridge, UK). The plasmid was cut simultaneously with the restriction endonucleases KpnI and PstI, as was the vector M13 mp19 (Bio-Rad). The linear form of M13 mp19 was ligated with the CaM coding segment followed by transformation into Escherichia coli strain MV1190 (Bio-Rad). The recombinants were

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\textsuperscript{1} The abbreviations used are: TnC, calcium binding component of troponin; CaM, calmodulin; CaM41/75, mutant of human liver calmodulin with Cys substituted for Gln\textsuperscript{41} and Lys\textsuperscript{75}; CaM85/112, mutant of human liver calmodulin with Cys substituted for Ile\textsuperscript{85} and Leu\textsuperscript{112}; DTT, dithiothreitol; HMJ model, Herzberg-Moult-James model.
screened by direct test for β-galactosidase activity and further confirmed by dideoxynucleotide sequencing (9).

Mutagenesis—In vitro site-directed mutagenesis was carried out using muta-gene M13 kit (Bio-Rad) following the method of Kunkel (10). To obtain CaM41/75 the CAG codon of Gin-41 and the AAA codon of Lys-75 were mutagenized simultaneously to the TGC codon of Cys. To obtain CaM46/112 the ATC codon of His6 and the TTA codon of Leu12 were mutagenized to the TGC codon of Cys. Selection and confirmation of the mutations were achieved by dideoxynucleotide sequencing (9).

Protein Expression and Purification—Originally the cDNA encoding the wild type human liver calmodulin or its mutants was cloned into the Ncol and PstI sites of the expression plasmid PK233-2, and then it was expressed in E. coli K3998. This expression system produced a relatively low yield of the mutant proteins. More recently the mutants were subcloned into pAE4D, the T7 expression vector/Phagemid (generous gift of Dr. Don S. Doering, Whitehead Institute, Cambridge, MA) at Ndel and PstI sites for the 5′ and 3′ ends, respectively, by polymerase chain reaction technique. Plasmids containing the mutants were transformed to a competent E. coli BL21 strain (Novagen), and cells were grown in 4 × 1 liter of LB medium at 37°C. Protein overexpression was induced with 0.3 mM isopropyl-1-thio-β-galactopyranoside when light scattering at 600 nm was 0.6–1.0. After another 2.5 h of incubation, bacteria were harvested by centrifugation (10,000 × g for 10 min). For protein purification a procedure based on that of Gopalakrishna and Anderson (11) has been used with modifications. The bacterial pellet was resuspended in a solution containing 50 mM Tris-HCl, pH 7.5, 2 mM EDTA, 1 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride (~60 ml/1 liter culture). The cell suspension was frozen in a dry-ice/methanol mixture and then thawed in a warm water bath. Freeze-thawing was repeated three more times, and cell debris were spun down for 30 min at 140,000 × g (in a Beckman 45 Ti rotor). The supernatant was collected, 5 mM CaCl2 was added, and the solution was applied at room temperature to a phenyl-Sepharose CL4B (Pharmacia Biotech Inc.) column (~50 ml of resin) equilibrated with a solution containing 50 mM Tris-HCl, pH 7.5, 5 mM CaCl2, 0.1 M NaCl, 1 mM EDTA. The column was washed with a solution containing 50 mM Tris-HCl, pH 7.5, 0.1 mM CaCl2, 1 mM DTT until absorption at 280 nm reached baseline. Then the column was rinsed with the same solution containing 0.5 M NaCl and finally with 150 ml of a solution containing 50 mM Tris-HCl, pH 7.5, 1 mM EGTA, 1 mM EDTA. The CaM-containing fractions were collected and applied to a DE-52 cellulose (Whatman) column (50 ml of resin) equilibrated with a solution containing 50 mM Tris-HCl, pH 7.5, 1 mM EGTA, 1 mM EDTA. The column was washed with 2–3 resin volumes of the equilibration buffer followed by a 0–0.6 M NaCl gradient in the same solution (2 × 110 ml). Fractions containing CaM (identified by SDS-polyacrylamide gel electrophoresis in the presence of 1 mM EDTA) were collected, dialyzed against 2 liters of solution containing 0.1 M NaCl and 2 mM NH4HCO3 followed by 4 × 6 liters of distilled H2O, and lyophilized.

Carboxymidomethylation—Lyophilized mutant CaM was dissolved in a solution containing 6 M urea, 0.1 M KCl, 0.2 M Tris-HCl, pH 8.0, 1 mM EDTA, 20 mM DTT and incubated for 1 h at 25°C. The sample was protected from light with aluminum foil and placed in an ice bath, and solid iodoacetamide was added in a 1:1 molar ratio with respect to the total SH content. After 20 min of incubation in dark at 0°C, DTT was added (final concentration, 20 mM), and the sample was dialyzed against a solution containing 0.1 M KCl, 20 mM Heps, pH 7.5, 2 mM EDTA.

Cyanylation—Mutant CaM (1–2 mg/ml) was treated with 10 mM DTT in a solution containing 6 M urea, 0.1 M KCl, 1 mM EDTA, 0.2 M Tris-HCl, pH 8.0, for 1 h at 25°C. Solid S-thiocyano-2-nitrobenzoic acid was then added at a 1:1 molar ratio with respect to the total amount of SH groups (prepared in a reaction with 5,5'-dithiobis(2-nitrobenzo-4-olate)). The sample was incubated for 1 h at 25°C and then dialyzed against a solution containing 0.1 M KCl, 20 mM Tris-HCl, pH 8.0, and finally against 20 mM Tris-HCl, pH 8.0. The extent of labeling (typically >90%) was estimated from native gel electrophoresis based on the observation that the unlabeled protein undergoes reoxidation and has higher mobility on the native gel.

Calcium Titration—Calcium titrations were performed in a solution containing 0.1 M KCl, 0.1 M Heps, pH 7.5, and 2 mM EGTA or 1 mM EGTA + 1 mM nitritrocitrate acid. Concentrations of free Ca2+ were calculated according to Ref. 12 using published values for the binding constants (13). The fluorescence data were fitted with the equation: F = F0 + ΔF [K(Ca2+)]γ/[1 + (K(Ca2+))]γ, where F0, ΔF, K, and γ are the initial fluorescence, the maximal fluorescence change, the apparent binding constant, and the Hill coefficient, respectively.

Enzyme Purification and Activity Assays—Purification of phosphodiesterase from bovine brain and the measurements of the Ca2+-CaM-dependent activity was performed according to Wallace et al. (14). For purification of calcineurin the procedure of Klee et al. (15) was used, and the activity was measured colorimetrically using p-nitrophenyl phosphate as substrate (16, 17). This method is less sensitive and requires higher concentration of the enzyme than those utilizing the 32P-labeled ATP but does not require the use of radioactivity.

Protein Concentration—Concentration of CaM and its mutants was estimated from UV absorbance using A280 (1%, 1 cm) = 1.6. Concentrations of phosphodiesterase and calcineurin were estimated by densitometric scanning of SDS-polyacrylamide gels using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Design of the Mutants—We have previously shown that the Ca2+-induced transition in the N-terminal domain of troponin C can be blocked by a disulfide bond between cysteine residues substituted for Gin68 in the B/C linker and Gin92 in helix D (18). Selection of the mutation sites was based on the proximity of the side chains in the Ca2+-free form and their large separation upon Ca2+-binding predicted by the HMJ model. Although the structure of the Ca2+-free N-terminal domain of CaM was not known, we expected it to be similar to that of TnC in view of the amino acid sequence homology and a similar secondary folding pattern. Therefore we have substituted cysteine residues for Gin84 and Lys95 (CaM41/75), the residues homologous to Gin68 and Gin92 of rabbit skeletal TnC.

It was less clear what residues should be mutated in the C-terminal domain because the Ca2+-free conformation of neither of the two proteins was known. We assumed that the Ca2+-free conformation of the C-terminal domain of CaM is similar to that of the N-terminal domain of TnC, in which case the F/G linker would undergo the largest displacement upon Ca2+-binding, and disulfide cross-linking of this segment to either helix H or helix E should be effective in blocking the transition. We have chosen the latter alternative (Fig. 1) and substituted Cys for Ile85 in helix E and for Leu112, which is located at the beginning of the F/G linker (CaM85/112), the selection being based on the observation that the side chains of the residues at the homologous positions in the Ca2+-free N-terminal domain of TnC are in contact.

Electrophoretic Mobility: Evidence for Intramolecular Disulfide Bonds—As expected, cysteine residues in both CaM41/75 and CaM85/112 readily form disulfide bonds that could be detected by an increase in the electrophoretic mobility of these proteins on polyacrylamide gels (Figs. 2 and 3). The disulfide in the N-terminal domain appears to be significantly more stable than that in the C-terminal domain because longer incubation with DTT is necessary for CaM41/75 than for CaM85/112 to obtain similar extent of reduction (Fig. 2). In the present studies the SH groups of the mutant proteins were either disulfide

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DISULFIDE CROSS-LINKED CALMODULIN MUTANTS

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**Fig. 1. Schematic representation of the mutant calmodulins showing positions of the disulfide cross-links.** Note that although each of the disulfide bonds appear to span a single EF-hand, most likely an entire two-site domain is blocked owing to strong interhelical interactions in the three-dimensional structure ( helices A/D, B/C, E/H, and F/G).
CaM mutants. Electrophoresis on 10% polyacrylamide gel in the presence of 80 mM Tris-Gly buffer, pH 8.6, and 1 mM EDTA. The gel for CaM41/75 contains also 4.5 mM urea. Prior to electrophoresis the protein samples (2–3 mg/ml) were incubated at 25 °C with 10 mM DTT in a solution containing 0.1 M NaCl and 50 mM Hepes, pH 7.5. The incubation was terminated by addition of excess of iodoacetamide. SH and S-S indicate the reduced and disulfide cross-linked forms of the CaM mutants, respectively.

**Fig. 3. Electrophoretic mobility of CaM mutants.** Electrophoresis on 10% polyacrylamide gel in the presence of 80 mM Tris-Gly buffer, pH 8.6, with or without 4.5 mM urea and 1 mM CaCl₂ or 1 mM EDTA as indicated. Lanes a, wild type human liver CaM; lanes b, ox-CaM41/75; lanes c, carboxyamidomethylated CaM41/75; lanes d, ox-CaM85/112; lanes e, carboxyamidomethylated CaM85/112; lanes f, cyanamidomethylated CaM85/112. Each lane contains 4 μg of protein.

**Fig. 2. Effect of DTT on the electrophoretic mobility of CaM mutants.** Electrophoresis on 10% polyacrylamide gel in the presence of 80 mM Tris-Gly buffer, pH 8.6, and 1 mM EDTA. The gel for CaM85/112 contains also 4.5 mM urea. Prior to electrophoresis the protein samples (2–3 mg/ml) were incubated at 25 °C with 10 mM DTT in a solution containing 0.1 M NaCl and 50 mM Hepes, pH 7.5. The incubation was terminated by addition of excess of iodoacetamide. SH and S-S indicate the reduced and disulfide cross-linked forms of the CaM mutants, respectively.

CaM41/75 CaM85/112

**Cross-linked or blocked by carboxyamidomethylation or cyanamidation to avoid uncontrolled oxidation. In Fig. 3 we compare effects of urea, Ca²⁺, and the blocking the cysteine residues on the electrophoretic mobility of CaM mutants. There is essentially no difference in electrophoretic mobility between CaM mutants and the wild type protein in the presence of Ca²⁺ regardless of the oxidation state of the Cys residues both in the presence and the absence of urea. However significant differences in mobility occur in the absence of Ca²⁺. The most striking is the large difference in mobility on a native gel between CaM85/112 having an intramolecular disulfide bond and that having the Cys residues blocked by carboxyamidomethylation or cyanamidation. It appears that the two forms of CaM85/112 have very different hydrodynamic properties; however, the nature of this difference is not clear.

**Calcium Binding—**We have examined the Ca²⁺ binding properties of CaM85/112 by monitoring tyrosine fluorescence. There are two tyrosine residues in CaM at position 99 and 138. Ca²⁺ binding to site III and IV of CaM results in a 2-fold increase in tyrosine fluorescence that provides a sensitive way of monitoring Ca²⁺ binding. The disulfide bond Cys⁸⁵–Cys¹¹² causes a large decrease in affinity for Ca²⁺ at sites III and IV as indicated by a shift of the titration curve to higher free [Ca²⁺] (Fig. 4). The titration curve becomes less steep (Hill coefficient n = 1.05), which may result from either a decrease in cooperativity or a difference in binding constants between the two sites. Because the disulfide connects the F/G linker with helix E, it is possible that site III is more affected and has lower affinity for Ca²⁺ than site IV. However fitting the titration data with two transitions (two independent sites with different binding constants) did not improve the fit over that obtained with a single transition (two sites with identical affinities), making it impossible to distinguish between the two models. Reduction of the disulfide with DTT and blocking the SH groups by their reaction with iodoacetamide restores the Ca²⁺ binding properties. In fact, there is an increase in affinity for Ca²⁺ as compared with the wild type CaM (Fig. 4).

The effect of the disulfide bond on the Ca²⁺ affinity of CaM85/112 is consistent with the HMJ model and can be explained on the basis of the coupling between Ca²⁺ binding and the opening of the interhelical interface in a two-EF-hand domain. Such coupling requires that the most favorable energetically interaction between Ca²⁺ and its ligands can be achieved only in the open conformation in which the two α-helices flanking the loop are perpendicular to each other. In CaM85/112 the disulfide bond precludes such a conformation, which results in less then optimal interaction with Ca²⁺ and a lower apparent binding constant. In the carboxyamidomethylated CaM85/112 there is no restriction of the helical movement. In addition there is a decrease in hydrophobic interactions between the helices in the Ca²⁺-free (closed) conformation due to the absence of Ile⁸⁵ and Leu¹¹². Thus the transition to the open conformation requires less energy, resulting in an increased affinity for Ca²⁺.

**Activation of Target Enzymes—**To evaluate the effect of the disulfide bonds on the ability of CaM mutants to activate target enzymes, phosphodiesterase and calcineurin were used. They represent two different classes of enzymes regulated by CaM distinguished by the predominant involvement of the N-terminal and C-terminal domains of CaM, respectively. We have used the oxidized forms of the CaM mutants, which have been further purified by reverse phase high pressure liquid chromatography to avoid possible interference with trace amounts of

![Image of electrophoretic mobility of CaM mutants](https://example.com/image1)

![Image of calcium binding properties](https://example.com/image2)
uncross-linked proteins not detectable by polyacrylamide gel electrophoresis. The disulfide cross-linked mutant calmodulins show no activity up to micromolar concentrations (Fig. 5), whereas the wild type CaM activates both enzymes in the nanomolar range. Our results indicate that it is sufficient to block either one of the two domains of CaM to completely inhibit its activating effect on both enzymes. Upon reduction of the disulfide bonds and blocking SH groups with iodoacetamide, the activation by CaM41/75 becomes similar to that by the wild type CaM; however, the activity of CaM85/112 is restored only partially (Fig. 5). Apparently this is due to the difference in the chemical character of the side chains at positions 85 and 112, for if the SH groups are blocked with CN groups in reaction with 2-nitro-5-thiocyanobenzoate, which is smaller and less hydrophilic than the carboxyamidomethyl group, the ability of the mutant to activate the target enzymes is restored to a significant degree (Fig. 5). We interpret these results as indicative of the involvement of the hydrophobic residues of Ile85 and Leu112 in the binding/activation of the target enzymes.

The loss of activity upon intradomain disulfide cross-linking of mutant CaM results, apparently, from a dramatic decrease in affinity for the target proteins because these mutants do not compete with the wild type CaM (data not shown). Although each of the two domains in CaM contributes one binding site for the target enzyme, no interaction occurs under experimental conditions when one of the domains is inaccessible. Such interpretation is consistent with the observation that proteolytic fragments of CaM comprising either half of the molecule are incapable of activating target enzymes (16). Our results indicate that the Ca\(^{2+}\)-induced opening of the structure in both domains of CaM is essential for the activation of targets by CaM.

Recently the three-dimensional structures of the complexes of calmodulin with synthetic peptides corresponding to the CaM-binding sites of myosin light chain kinase from skeletal (19) and smooth muscle (20) as well as CaM-dependent kinase II (21) have been reported. Unlike the free CaM, the complex has a compact globular structure. The two domains of CaM (residues 6–73 and 83–146) remain essentially unchanged; however, the long central helix is disrupted into two helices connected by a long flexible loop, thereby enabling the two domains to clamp the bound peptide that adopts a helical conformation. In this respect the structure is similar to the model proposed earlier by Perschini and Kretsinger (22). The peptide is located in a hydrophobic channel formed by the hydrophobic faces of the two globular domains of CaM (for review see Ref. 23). It is clear from these structures that the disulfide bonds in CaM41/75 and CaM85/112 would preclude the formation of such complexes consistent with our data.

After this work was completed two independent reports were published on the three-dimensional structure of Ca\(^{2+}\)-free calmodulin determined by multidimensional heteronuclear NMR (24, 25). Also the Ca\(^{2+}\)-bound and Ca\(^{2+}\)-free structures of the C-terminal domain fragment of calmodulin (26) and the N-terminal domain of troponin C (27) were determined by NMR. These studies show that both domains of CaM and the N-terminal domain of TnC undergo a Ca\(^{2+}\)-induced transition consistent with the HMJ model. The distances between C\(_\beta\) atoms of Gln41 and Lys75 and between C\(_\beta\) of Ile85 and Leu112 calculated from the averaged atomic coordinates of the Ca\(^{2+}\)-free CaM (25) are 7.8 and 6.9 Å, respectively. This is slightly longer than the 3.4–4.2 Å range predicted to be optimal for a disulfide bond formation (28). However, the segments of the polypeptide chain at all four mutation sites show significant flexibility (24–26); thus it is unlikely that any significant long range structural alterations would be necessary for the disulfide bonds to form in CaM41/75 and CaM85/112. The corresponding distances in the Ca\(^{2+}\)-bound conformation are 17.1 and 16.4 Å in the N- and C-terminal domains, respectively, which clearly would be impossible in the presence of the disulfide bonds.

Our present data and the recent NMR studies indicate that the transition between the closed and open conformation in each of the two domains of CaM consistent with the HMJ model (4) represents the key Ca\(^{2+}\)-induced regulatory transition in this protein. Most likely it is a general mechanism of Ca\(^{2+}\) regulation in proteins of the EF-hand family. This applies only to the so-called “regulatory” proteins such as CaM and TnC, but presumably not to those referred to as Ca\(^{2+}\) buffers. In the latter case exemplified by calbindin D\(_{9K}\), there is no Ca\(^{2+}\)-induced increase in hydrophobicity and no change in interhelical angles (29). At this point it is not clear what structural features are responsible for the tight coupling between Ca\(^{2+}\) binding and the conformational transitions in Ca\(^{2+}\) regulators and the lack of it in Ca\(^{2+}\) buffers.

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REFERENCES
1. Babu, Y. S., Sack, J. S., Greenhough, T. J., Bugg, C. E., Means, A. R., and Cook, W. J. (1985) Nature 315, 37–40
2. Herzberg, O., and James, N. G. (1985) Nature 313, 653–659
3. Sundaralingam, M., Bergstrom, R., Strasburg, G., Rao, S. T., Roychowdhury, W. J. (1985) Nature 315, 37–40
P., Greaser, M., and Wang, B. C. (1985) Science 227, 945–948
4. Herzberg, O., Moult, J., and James, M. N. (1986) J. Biol. Chem. 261, 2638–2644
5. Grabarek, Z., Tao, T., and Gergely, J. (1992) J. Muscle Res. Cell Motil. 13, 383–393
6. Gagne, S. M., Tsuda, S., Li, M. X., Chandra, M., Smillie, L. B., and Sykes, B. D. (1994) Protein Sci. 3, 1961–1974
7. Strynadka, N. C. J., Chernai, M., Li, M., Smillie, L. B., and James, M. N. G. (1995) Biophys. J. 68, A359
8. Wawrzyniak, E. J., and Perham, R. N. (1984) Biochem. Int. 9, 177–185
9. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 5463–5467
10. Kunkel, T. A. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 488–492
11. Gopalakrishna, R., and Anderson, W. B. (1982) Biochem. Biophys. Res. Commun. 104, 830–836
12. Perrin, D. D., and Sayco, J. G. (1967) Talanta 14, 833–842
13. Sillen, L. C., and Martel, A. E. (1964) Stability Constants of Metal Ion Complexes, 2nd Ed., The Chemical Society, Burlington House, London
14. Wallace, R. W., Tallant, E. A., and Cheung, W. Y. (1983) Methods Enzymol. 102, 39–47
15. Klee, C. B., Krinks, M. H., Manalan, A. S., Cohen, P., and Stewert, A. A. (1983) Methods Enzymol. 102, 227–244
16. Newton, D., Oldewurtel, M. D., Krinks, M. H., Shiloch, J., and Klee, C. B. (1985) J. Biol. Chem. 259, 4419–4426
17. Pallen, C. J., and Wang, J. H. (1983) J. Biol. Chem. 258, 8550–8553
18. Grabarek, Z., Tan, R. Y., Wang, J., Tao, T., and Gergely, J. (1990) Nature 345, 132–135
19. Ikura, M., Clore, G. M., Gronenborn, A. M., Zhu, G., Klee, C. B., and Bax, A. (1992) Science 256, 632–638
20. Meador, W. E., Means, A. R., and Quiocio, F. A. (1992) Science 257, 1251–1255
21. Meador, W. E., Means, A. R., and Quiocio, F. A. (1993) Science 262, 1718–1721
22. Persechini, A., and Kretzinger, R. H. (1988) J. Cardiovasc. Pharmacol. 12, 51–512
23. Clore, G. M., Bax, A., Ikura, M., and Gronenborn, A. M. (1993) Curr. Opin. Struct. Biol. 3, 838–845
24. Zhang, M., Tanaka, T., and Ikura, M. (1995) Nat. Struct. Biol. 2, 758–767
25. Kuboniwa, H., Tjandra, N., Grzesiek, S., Ren, H., Klee, C. B., and Bax, A. (1995) Nat. Struct. Biol. 2, 768–776
26. Finn, B. E., Evanas, J., Drakenberg, T., Walther, J. P., Thulin, E., and Forsen, S. (1995) Nat. Struct. Biol. 2, 777–783
27. Gagne, S. M., Tsuda, S., Li, M. X., Smillie, L. B., and Sykes, B. D. (1995) Nat. Struct. Biol. 2, 784–789
28. Sowdhamini, R., Srinivasan, N., Shoichet, B., Santi, D. V., Ramakrishnan, C., and Balaram, P. (1989) Protein Eng. 3, 95–103
29. Skelton, N. J., Kordel, J., Akke, M., Forsen, S., and Chazin, W. J. (1994) Nat. Struct. Biol. 1, 239–245
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