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Activation of *Bordetella pertussis* Adenylate Cyclase by the Carboxyl-Terminal Tryptic Fragment of Calmodulin

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ABSTRACT: Highly purified tryptic fragments of calmodulin were tested for their ability to stimulate adenylate cyclase activity of *Bordetella pertussis* spheroplast membranes and were compared to their activities on brain Ca^{2+}/calmodulin-dependent cyclic nucleotide phosphodiesterase. The C-terminal fragment, consisting of residues 78–148, was a full agonist for the cyclase with 0.1–0.15 the potency of calmodulin but did not stimulate phosphodiesterase. Fragments 1–77, 1–90, and 107–148 stimulated adenylate cyclase (and not phosphodiesterase) at low potency; this was not due to calmodulin contamination, but contamination by fragment 78–148 could not be excluded with certainty. An adduct of norchlorpromazine isothiocyanate and calmodulin showed full agonist activity for adenylate cyclase at 0.01–0.02 the potency of calmodulin. Stimulation of adenylate cyclase by a number of the fragments occurred in the absence of Ca^{2+}, but stimulator potency was enhanced 20–60-fold in its presence. The similarity of Ca^{2+} requirements of fragment 78–148 and calmodulin suggests that occupancy of the two C-terminal Ca^{2+} binding sites of calmodulin accounts for most of the Ca^{2+} enhancement of calmodulin stimulation of adenylate cyclase.

Many enzymes are activated by calmodulin (Klee & Vanaman, 1982), and it has often been assumed that the mode of this activation is based on a similar calmodulin binding domain in these otherwise different target proteins. However, a number of the characteristics of the binding interaction show wide variations from one protein to the next: (1) the dissociation constants for calmodulin, usually in the nanomolar range, are much higher for MAP and τ (Lee & Wolff, 1984), spectrin/fodrin (Davies & Klee, 1981; Sobue et al., 1980), and troponin I (Keller et al., 1982); (2) Ca^{2+} is an obligatory participant in the reaction of calmodulin with most enzymes but is not required for interaction with phosphorylase kinase (Cohen et al., 1978), histones (Khandelwal et al., 1980), troponin I (Olwin et al., 1982), a brush border protein (Glenney & Weber, 1980), protein P-57 (Andreasen et al., 1983), and the adenylate cyclases of *Bordetella pertussis* (Greenlee et al., 1982; Kilhoffer et al., 1983) and *Bacillus anthracis* (Leppla, 1985).

An alternative approach to an understanding of such differential effects has been with the use of calmodulin derivatives. On the one hand, chemical modifications have led to differential effects on a number of calmodulin-sensitive enzymes (Thiry et al., 1980). On the other hand, limited proteolytic cleavage by trypsin or thrombin has yielded fragments containing residues 1–77 and 78–148, 1–90, 1–106, and 107–148, retaining one, two, or three of the Ca^{2+} binding domains (Drabikowski et al., 1977). Some of these can still activate certain enzymes but have lost all ability to activate phosphodiesterase and myosin kinase (Drabikowski et al., 1982; Guerini et al., 1984; Kuznicki et al., 1981; Newton & Klee, 1984; Newton et al., 1984, 1985; Wall et al., 1981; Walsh et al., 1977). Because the fragments have much lower affinities for the target proteins than calmodulin, it is important that contamination by calmodulin be $\ll$0.1%. Earlier studies were ambiguous because low levels of contamination by intact calmodulin could not be ruled out. Several recent studies have succeeded in overcoming this objection by substantial refinements in purification and detection methods (Guerini et al., 1984; Newton et al., 1984, 1985). Using these criteria, cyclic nucleotide phosphodiesterase and myosin kinase can now be shown not to be stimulated by any of the proteolytic fragments in concentrations up to 1×10^{-5} M. This stringent requirement for the intact regulator thus made the activation of phosphodiesterase (PDE)[1] a reliable detection system for contamination by calmodulin, and we have used tryptic calmodulin fragments so tested to assess their ability to activate the adenylate cyclase of *B. pertussis*.

Bordetella pertussis organisms contain a very active extracellular adenylate cyclase that is largely confined to the

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[1] Abbreviations: PDE, phosphodiesterase; AC, adenylate cyclase; CaM, calmodulin; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; $CAPP_1$–calmodulin, 1 to 1 covalent adduct of 2-chloro-10-(3-aminopropyl)phenothiazine isothiocyanate with calmodulin; kDa, kilodalton(s); Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

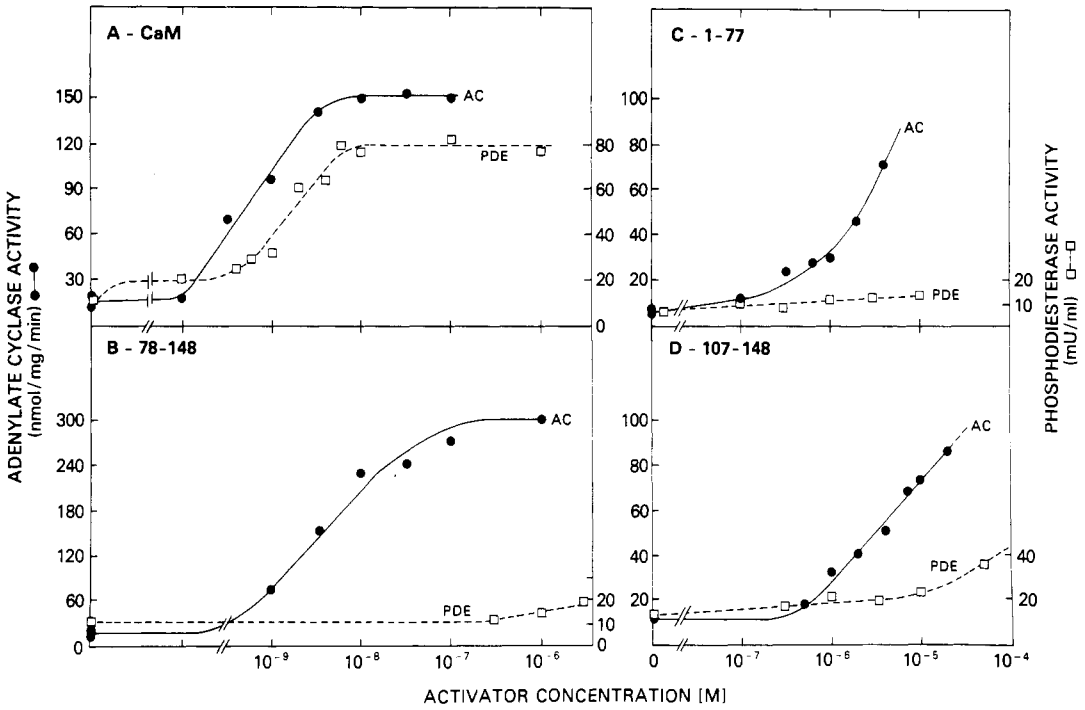

FIGURE 1: Dose–response curves of adenylate cyclase of *B. pertussis* spheroplast membranes and purified phosphodiesterase from brain by calmodulin and its proteolytic fragments. Cyclase (AC) was assayed for 10 min at 30 °C with 5 mM ATP, 10 mM Mg^{2+}, and 38.8 μM Ca^{2+}. Free metal concentrations were 5.1 mM Mg^{2+} and 26.9 μM Ca^{2+}. Phosphodiesterase (PDE) was assayed for 40 min at 30 °C as described (Newton et al., 1985). Reactions were started by enzyme addition. (A) Bovine testis calmodulin; (B) the carboxy-terminal half consisting of residues 78-148; (C) the amino-terminal half consisting of residues 1–77; (D) residues 107–148 containing only the fourth Ca^{2+} binding domain.

periplasmic space and is readily accessible to external ATP and regulators (Hewlett et al., 1976; Hewlett & Wolff, 1976). It is composed of a single subunit of 47 kDa, is not modulated by GTP or related nucleotides, and has a very high turnover number (Wolff, 1985). It is activated by calmodulin from various sources (Goldhammer & Wolff, 1982; Greenlee et al., 1982; Kilhoffer et al., 1983; Wolff et al., 1980) but not by other Ca^{2+} binding proteins such as troponin C or parvalbumin. Calcium enhances the sensitivity of the cyclase to calmodulin but is not an absolute requirement for activation (Greenlee et al., 1982; Kilhoffer et al., 1983). The absence of a Ca^{2+} requirement suggested the possibility that this enzyme is also less stringent than phosphodiesterase in its requirement for the intact calmodulin molecule. We show here that the cyclase responds to a variety of calmodulin fragments that could not activate the phosphodiesterase at the same concentrations.

Experimental Procedures

Spheroplast membranes from *Bordetella pertussis* organisms (strain 114) were prepared as previously described (Goldhammer & Wolff, 1982); 50- or 100-μL aliquots were stored at −20 °C and diluted with 20 mM Tris-HCl buffer, pH 7.9, with the aid of a hand-operated glass homogenizer just before use. Adenylate cyclase was assayed at 30 °C for 10 min by addition of 8–10 μg of membrane protein to a prewarmed medium composed of 5.0 mM ATP, 10 mM $MgCl_2$, 10 μM added $CaCl_2$, 60 mM Tris-HCl buffer, pH 7.9, and 0.5–1.0 μCi of [α-^{32}P]ATP per tube (800 mCi/μmol from NEN) unless otherwise noted in the legends. All calmodulin or fragment dilutions were made in a solution of 0.01% bovine serum albumin that had been previously found to have a negligible calmodulin contamination. Reactions were stopped by addition of 100 μL of a solution containing 20 mM ATP, 6.5 mM cAMP, and ~6000 cpm of [^{3}H]cAMP in 1% sodium dodecyl sulfate, processed according to Salomon et al. (1974), and counted to a statistical error of <1.5%.

Membrane protein was determined by the bicinchoninic acid method as supplied by the Pierce Chemical Co.

Tryptic fragments of bovine testis calmodulin were prepared as described previously (Newton et al., 1984). The covalent adduct of calmodulin and norchlorpromazine (called $CAPP_1$–calmodulin) was prepared as described (Newton et al., 1983). Fragment purity and phosphodiesterase assays were carried out as described (Newton et al., 1984). In a number of preparations, no phosphodiesterase stimulating or blocking activities could be discerned at 1×10^{-5} M; hence, comparisons with adenylate cyclase were made at this or at lower concentrations of the fragments.

The complete incubation mixture (minus the [α-^{32}P]ATP) contained 28.8 ± 2 μM total calcium by atomic absorption measurements. Under standard assay conditions (see above), the calculated free Ca^{2+} concentration is 26.9 μM and free Mg^{2+} concentration 5.1 mM. Calcium titrations were carried out in the presence of 50 μM EGTA (Figure 4). Free Ca^{2+} concentration was calculated according to a program for multiple metals and ligands according to Fabiato and Fabiato (1979). We are grateful to Dr. Thiery Jean for use of his program.

Results and Discussion

A comparison of the activation of *B. pertussis* adenylate cyclase and phosphodiesterase by calmodulin and selected proteolytic fragments is depicted in Figure 1. It is clear that the responsiveness of both enzyme activities to intact calmodulin is of the same order, the spheroplast membranes being slightly more sensitive (Figure 1A). However, here the similarity in response ends. None of the fragments tested were able to activate the phosphodiesterase at concentrations of $\leq 1 \times 10^{-5}$ M, as previously reported (Newton et al., 1984), whereas the adenylate cyclase proved to be far less fastidious with respect to activation by calmodulin fragments and responded over a wide range of concentrations from nanomolar

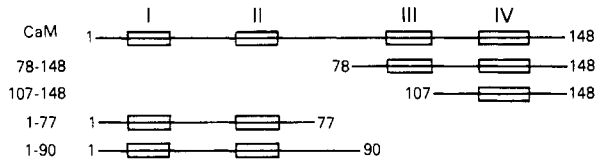

FIGURE 2: Diagram of the tryptic fragments of calmodulin used in this study and the location of the Ca^{2+} binding domains (depicted as rectangles).

to micromolar. The highest activity was obtained with the C-terminal fragment, composed of residues 78–148 (Figure 1B), which is believed to contain the two high-affinity calcium binding domains III and IV (Klee et al., 1986; Minowa & Yagi, 1984; Thulin et al., 1984). It was ~0.13 as potent as the intact calmodulin and was a full agonist (Figure 1B). The average concentration required for half-maximal stimulation by fragment 78–148 for all experiments was 5 nM compared to 0.7 nM for calmodulin. This can clearly not be accounted for by contamination as it would easily have been detected by physical methods as well as by the ability to activate phosphodiesterase. The greater activation produced by fragment 78–148 (compare Figure 1A and Figure 1B) may be ascribed to the use of different membrane preparations. However, with the same membranes, fragment 78–148 sometimes yielded greater maximal stimulation than calmodulin did. The basis for this difference is not known at present. Addition of maximal concentrations of calmodulin to maximal concentrations of fragment 78–148 shows no additivity. Thus, 0.33 μM calmodulin, 1 μM fragment 78–148, or both added together to the membrane preparation yielded rates of cAMP generation of 92, 104, and 94 nmol mg^{-1} min^{-1}, respectively. The high potency of the fragment may be related to the remarkable preservation of structural features of the native molecule as evidenced by good Ca^{2+} affinity and Ca^{2+}-induced alterations in the proton NMR spectra (Thulin et al., 1984; Ikura et al., 1984; Klee et al., 1986) and the "normal" secondary structure and its changes with Ca^{2+} (Drabikowski et al., 1982). The fact (1) that there was no activation of the phosphodiesterase when the cyclase was fully activated (Figure 1B) and (2) that fragment 78–148 was the only fragment tested (Newton et al., 1984) that acted as a competitive inhibitor of calmodulin on phosphodiesterase (K_i = 1.5 μM) suggests that despite the close resemblance to the native protein, activation of the phosphodiesterase requires participation of the N-terminal half of the molecule whereas the cyclase does not.

A schematic drawing of the linear sequence of calmodulin and the tryptic fragments used in this study is depicted in Figure 2. Also indicated is the location of the four calcium binding domains sequentially numbered I–IV. Calmodulin fragments containing calcium binding sites I and II, residues 1–77 (Figure 1C) or 1–90 (Figure 3), activate the adenylate cyclase with nearly equal potency, but enough material was not available to attain saturation (5-fold stimulation is attained at 0.9 μM). As is true for fragment 78–148, the N-terminal fragment (1–77) retains a stable folded conformation (Anderson et al., 1985; Drabikowski et al., 1982; Thulin et al., 1984). For example, the circular dichroic spectrum of 1–77 is preserved, shows nearly the same calcium transition midpoint as calmodulin, and when added to that of fragment 78–148 yields essentially the same spectrum as the native molecule. Incidentally, this observation suggests the lack of major interactions between the N-terminal and C-terminal domains in the intact molecule. Nevertheless, fragment 1–77 is much less active than fragment 78–148. As found with fragment 1–77, activation of *B. pertussis* adenylate cyclase by fragment

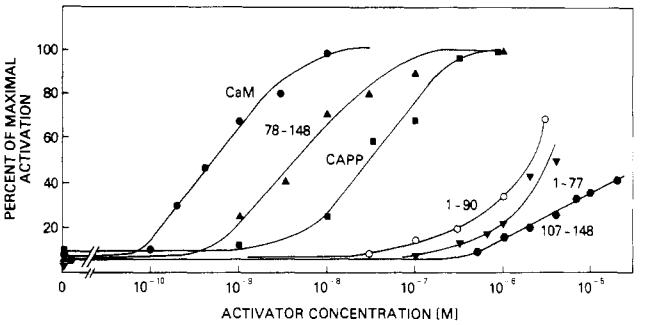

FIGURE 3: Comparison of calmodulin fragments in the activation of *B. pertussis* adenylate cyclase. Calmodulin (CaM), fragment 78–148, and $CAPP_1$–calmodulin (CAPP) were normalized to 100% and had maximal values of 246, 301, and 175 nmol mg^{-1} min^{-1}, respectively. For better visualization, activities of fragments 1–77, 1–90, and 107–148 were multiplied by 1.5, 2.0, and 1.5, respectively. Conditions as for Figure 1.

1–90 (Figure 3) can not be due to calmodulin contamination because concentrations as high as 1×10^{-5} M do not activate phosphodiesterase (data not shown). However, contamination by fragment 78–148 would have to be <0.3%, and this cannot be assured in the preparation used. Apparently, amino acid residues 78–90, which are part of the long central helix (helix IV), do not, by themselves, contribute significantly to the activity residing in the N-terminal half of the calmodulin since the 13-residue extension did not alter potency.

Assessment of the potency of fragment 1–106, which might be expected to be more potent than 1–90 since it contains the third calcium binding site though not all of its C-terminal α-helix, led to curves that were superimposable on the fragment 1–90 dose–response curves (data not shown). Since this material was, however, contaminated with ~0.1% intact calmodulin, as judged by phosphodiesterase assays, it is impossible to ascribe such stimulation to intrinsic activity (results not shown).

Results for adenylate cyclase assays on fragment 107–148, which contains only calcium binding domain IV, are shown in Figure 1D. *B. pertussis* adenylate cyclase shows activation in the low micromolar range. A low level of phosphodiesterase activity is observed only at the highest concentration of the fragment (5×10^{-5} M) which is equivalent to ~0.002% contamination with intact calmodulin. Fivefold stimulation occurring at 6×10^{-6} M for adenylate cyclase would thus be equivalent to 1.2×10^{-10} M calmodulin (or a 0.02% calmodulin contamination). Such concentrations of calmodulin do not stimulate 5-fold in these membrane preparations; hence, there appears to be activity in this one-domain calmodulin fragment provided there is no significant contamination by fragment 78–148.

Attempts to induce complementation with the N-terminal and C-terminal fragments were carried out with the simultaneous addition of 1×10^{-7} M fragment 1–77 and 1×10^{-9} M fragment 78–148. We found only simple additivity and no return to the level of activation of the cyclase expected from 1×10^{-9} M intact calmodulin. Addition of 2 μM fragment 107–148 to 1 μM fragment 1–106 yielded less than additive cyclase activity. These negative findings are consistent with those obtained for phosphodiesterase (Newton et al., 1984). However, optimal conditions for reassociation have not been investigated.

An additional derivative of calmodulin, a covalent adduct of norchlorpromazine isothiocyanate and calmodulin called $CAPP_1$–calmodulin (Newton et al., 1983, 1985; Newton & Klee, 1984), was assayed with *B. pertussis* adenylate cyclase. This derivative is a calmodulin antagonist of cyclic nucleotide

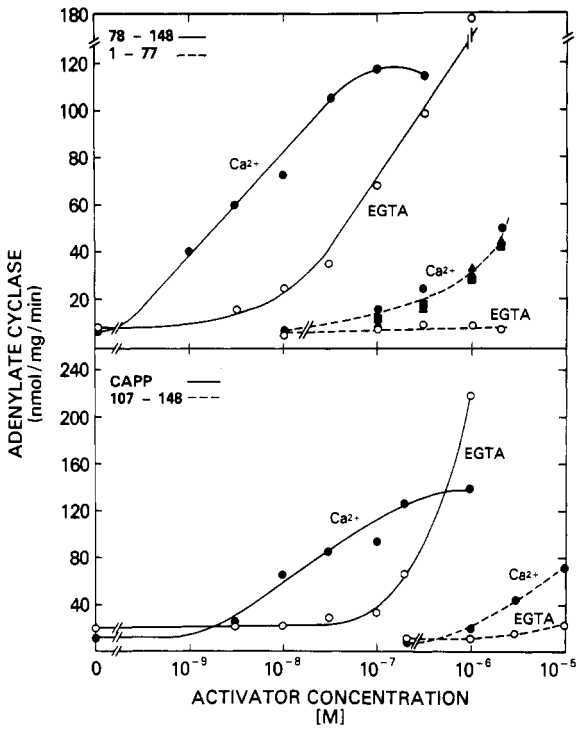

FIGURE 4: Ca^{2+} requirement of *B. pertussis* adenylate cyclase stimulation by calmodulin derivatives. Assay conditions for Ca^{2+} media (●) as in Figure 1. EGTA was present at a total concentration of 2.0 mM (○). The free Ca^{2+} concentrations were 26.9 and 0.0004 μM for the Ca^{2+} and EGTA media, respectively. For experiments with fragment 1–77, free Ca^{2+} concentrations were 26.9 (●), 40.9 (▲), and 89.7 (■) μM.

phosphodiesterase and myosin light chain kinase activation but a partial agonist of calcineurin. Figure 3 shows that $CAPP_1$–calmodulin is a full agonist for adenylate cyclase activation with a potency of 0.02 of native calmodulin and half-maximal stimulation at 4×10^{-8} M. Attempts to demonstrate a competitive effect with calmodulin showed only simple additivity. Three other calmodulin-sensitive enzymes have been shown to be activated by $CAPP_1$–calmodulin: glycogen synthase kinase and phosphorylase kinase (Newton et al., 1985) and plasma membrane ATPase (J. Krebs and E. Carafoli, personal communication). The derivative shows potencies of 0.72, 0.32, and 0.13 of the unmodified calmodulin, respectively, and thus is a more potent agonist for these enzymes than for the adenylate cyclase.

It has been shown previously that the adenylate cyclase of *B. pertussis* organisms could be activated by calmodulin even in the presence of EGTA, albeit at lower potency (Greenlee et al., 1982; Kilhoffer et al., 1983). It was thus of interest to learn whether this property persisted in the proteolytic fragments. Like the intact molecule of calmodulin, a number of the derivatives tested stimulated in the absence of Ca^{2+} for *B. pertussis* adenylate cyclase (Figure 4). Without Ca^{2+}, the extent of activation produced by fragment 78–148 was comparable to that with Ca^{2+}, but a 20–60-fold greater concentration was required than in the presence of calcium. The calculated free Ca^{2+} concentrations were $(3–6) \times 10^{-10}$ M in the presence of 2 mM EGTA and 26.9 μM in its absence. With fragment 1–77, not enough material was available to check if activation would have occurred in the presence of EGTA (Figure 4). This was also true for fragment 1–90 in the presence of EGTA (data not shown). Because of the lower Ca^{2+} affinity of the N-terminal half of the molecule (Klee et al., 1986; Minowa & Yagi, 1984), we tested higher concentrations of Ca^{2+} (40.9 and 89.7 μM) in the assay. This did not change the extent of cyclase stimulation (Figure 4). On the other hand, high enough concentrations of fragment 107–148 did produce some activation in the presence of EGTA (Figure 4). As shown with fragment 1–77, increasing the Ca^{2+} concentration above those of the basal mixture (26.9 μM) yielded no further enhancement of activity (data not shown). Since higher Ca^{2+} concentrations inhibit *B. pertussis* adenylate cyclase (Hewlett & Wolff, 1976), the full range of Ca^{2+} concentrations could not be tested. As with native calmodulin, activation by $CAPP_1$–calmodulin was shifted by about 20-fold in the presence of 2 mM EGTA.

As we have shown previously (Kilhoffer et al., 1983), maximal stimulation by calmodulin is greater in the presence of EGTA than in its absence. This phenomenon is also observed with fragment 78–148 and with $CAPP_1$–calmodulin. This overshoot may be ascribed to chelation of a metal other than Ca^{2+} (or Mg^{2+}) because it can also be observed upon exposure to 1,10-phenanthroline (Kilhoffer et al., 1983).

For a number of years, discrepancies have been noted between direct measurements of Ca^{2+} binding to calmodulin, which yield a nearly continuous function, and the stepwise spectroscopic transitions occurring with Ca^{2+} saturation. The use of fragments 1–77 and 78–148 containing sites I and II and sites III and IV, respectively (see Figure 2), failed to resolve this since both halves of the molecule yielded similar affinities for Ca^{2+} (Klee et al., 1986; Minowa & Yagi, 1984). In the presence of Mg^{2+}, however, differences between the low- (1–77) and high-affinity (78–148) domains of calmodulin could be detected (Klee et al., 1986), providing strong support for the cooperative model of Wang (1985). The sensitivity to Mg^{2+} appears to require the intact calmodulin molecule (Klee et al., 1986). It was important to ascertain whether the high Mg^{2+} levels used in our cyclase assay might contribute significantly to the relative potencies of the fragments. Because Mg^{2+} is part of the substrate, it could not be deleted. Accordingly, we have compared 1 and 10 mM Mg^{2+} in the presence of 0.5 mM ATP at a fixed activator concentration. Titration of adenylate cyclase activation as a function of free Ca^{2+} concentration reveals (Figure 5) that half-maximal Ca^{2+} concentrations are increased 70–80% when the Mg^{2+} concentration is increased from 1 to 10 mM (total) or from 0.55 to 9.5 mM free Mg^{2+}—for calmodulin, the shift is from 9.5 to 16 μM Ca^{2+}, and for fragment 78–148, it is from 11.8 to 19 μM Ca^{2+}. This similarity in the Mg^{2+}-dependent shift in the Ca^{2+} activation curves suggests that the negative cooperative effect of Mg^{2+} seen in intact calmodulin (Klee et al., 1986; Minowa & Yagi, 1984) is not a major determinant in the response of the adenylate cyclase but reflects primarily competition by Mg^{2+} for the Ca^{2+} binding sites (Haiech et al., 1981). This suggests that occupancy of the two C-terminal sites is sufficient to specify the Ca^{2+}-dependent portion of the activation of the *B. pertussis* adenylate cyclase by intact calmodulin.

CONCLUSIONS

A summary of published results using proteolytic fragments of calmodulin as enzyme activators is given in Table I. It is apparent that cyclic nucleotide phosphodiesterase and the bacterial adenylate cyclase are at the extreme ends of stringency for native calmodulin. Like calmodulin, fragment 78–148 and $CAPP_1$–calmodulin are full agonists for adenylate cyclase whose activities cannot be accounted for by calmodulin contamination. Unlike all other enzymes tested so far, the potency for adenylate cyclase is much less affected by protein cleavage than by phenothiazine substitution. Like calmodulin (Greenlee et al., 1982; Kilhoffer et al., 1983), neither fragment

Table I: Biological Activities of Calmodulin Fragments

enzyme	calmodulin fragments[a] 1–77	1–106	78–148	107–148	$CAPP_1$–CaM	comment	ref[b]
phosphodiesterase	0	0	–	0	–	1–77 and 78–148 bind	a–d
							a–c
calcineurin	0	0	0	0	±	partial agonist ($CAPP_1$–CaM)	b, c, e
phosphorylase kinase	+ (0.3)		+ (5)		+ (32)		c, e
glycogen synthase kinase	0		0		+ (72)		e
myosin light chain kinase	0		–		–	K_i(78–148) = 8×10^{-7} M	b, c, e
Ca^{2+}-ATPase	0	+ (0.1)	+ (0.8)		+ (13)		f
adenylate cyclase	+ (0.45)	?+	+ (13)	+ (<0.1)	+ (1.0)		

[a] (+) indicates activation; (–) indicates inhibition; (0) indicates tested but no effect. Numbers in the parentheses indicate approximate percent of calmodulin potency. [b] The following references are cited: (a) Klee et al. (1986); (b) Newton et al. (1985); (c) Newton et al. (1984); (d) Ni & Klee (1985); (e) Newton & Klee (1984); (f) Guerini et al. (1984).

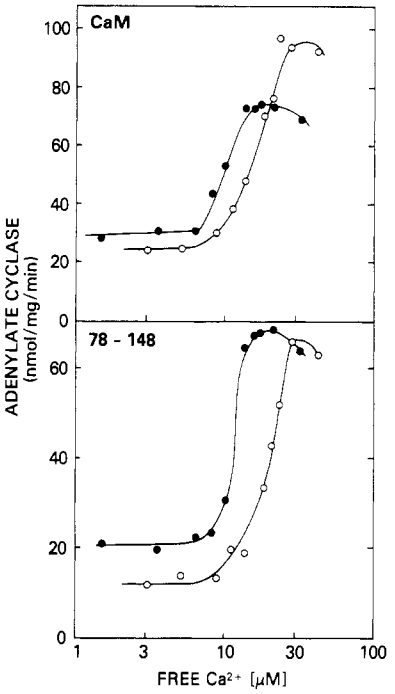

FIGURE 5: Effect of Mg^{2+} on the Ca^{2+} titration of adenylate cyclase activity in the presence of calmodulin or fragment 78–148. Assays were carried out with 0.5 mM ATP, 50 μM EGTA, and either 1 mM (●) or 10 mM (○) total Mg^{2+} (or 0.55 and 9.5 mM free Mg^{2+}, respectively). Concentrations of activators were 3×10^{-8} M calmodulin (CaM) or 6×10^{-8} M fragment 78–148. The reaction was carried out under otherwise standard conditions and was started with 8–10 μg of *B. pertussis* spheroplast membrane protein. Half-maximum free Ca^{2+} concentrations at low and high Mg^{2+} levels were 9.5 and 16 μM for CaM and 11.8 and 19 μM for fragment 78–148.

78–148 nor $CAPP_1$–calmodulin has an absolute requirement for Ca^{2+} in the activation of *B. pertussis* adenylate cyclase (Figure 4). Thus, in view of the considerable sequence homology between the Ca^{2+} binding domains (Klee & Vanaman, 1982; Watterson et al., 1980), some simple structural feature of the helix–loop–helix arrangement present in both Ca^{2+}-loaded and Ca^{2+}-free conformations of calmodulin may suffice for activation of *B. pertussis* adenylate cyclase. Nevertheless, a Ca^{2+} effect persists as shown by a marked increase in calmodulin affinity for the enzyme that occurs at Ca^{2+} concentrations of 9 μM for half-maximal activation at 1 mM Mg^{2+}. A similar affinity change is seen with exposure of fragment 78–148 to Ca^{2+} (half-maximal at 12 μM). This suggests to us that the Ca^{2+} effect on this cyclase, seen with intact calmodulin, may well be explained by Ca^{2+} occupancy of only the two C-terminal binding sites.

The *B. pertussis* adenylate cyclase is also stimulated by tryptic fragments 1–77, 1–90, and 107–148. Although very much less potent than calmodulin or fragment 78–148, the activation is not due to contamination by calmodulin. Unfortunately, contamination by fragment 78–148 is much more difficult to rule out for lack of an independent biological test and the insensitivity of chemical assays. The question of intrinsic activity of these fragments must be left in abeyance.

In view of the less stringent structural requirements for the activation of adenylate cyclase, it is perhaps surprising that troponin C, which shows substantial sequence homology as well as crystallographic similarities, is not an activator (Wolff et al., 1980). Similarly, parvalbumin, which shares many structural features with calmodulin, does not activate the cyclase (Kilhoffer et al., 1983). It has been suggested that the sharper interhelix angles about the Ca^{2+} binding domains of calmodulin as compared to parvalbumin or troponin C (Babu et al., 1985; Herzberg & James, 1985) might account for some functional differences between these proteins. Whether this applies to adenylate cyclase activation is not known at present. Yet some differences in the cyclase stimulations by intact bovine brain, peanut, and *Tetrahymena* calmodulins have been reported (Goldhammer & Wolff, 1982; Wolff et al., 1980). It appears, therefore, that while intrinsic activating properties for *B. pertussis* adenylate cyclase can be assigned to some individual Ca^{2+} binding domains, there is a significant enhancement of potency when these are combined in the intact protein which, in turn, is subject to regulation by small changes in the primary sequence.

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Registry No. Ca, 7440-70-2; adenylate cyclase, 9012-42-4.

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