# $Ca^{2+}$-Dependent Cyclic Nucleotide Phosphodiesterase Is Activated by Poly(L-aspartic acid)†

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ABSTRACT: $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase ($Ca^{2+}$-PDE) activity was stimulated by poly(L-aspartic acid) but not by poly(L-glutamic acid), poly(L-arginine), poly(L-lysine), and poly(L-proline). This activation was $Ca^{2+}$ independent and did not further enhance the activation of $Ca^{2+}$-PDE by $Ca^{2+}$–calmodulin (CaM). Poly(L-aspartic acid) produced an increase in the $V_{max}$ of the phosphodiesterase, associated with a decrease in the apparent $K_m$ for the substrate, such being similar to results obtained with $Ca^{2+}$–CaM. Poly(L-aspartic acid) did not significantly stimulate myosin light chain kinase and other types of cyclic nucleotide phosphodiesterase. CaM antagonists such as *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), trifluoperazine, and chlorpromazine selectively antagonized activation of the enzyme by poly(L-aspartic acid). Kinetic analysis of W-7-induced inhibition of activation of phosphodiesterase by poly(L-aspartic acid) was in a competitive fashion, and the $K_i$ value was 0.19 mM. On the other hand, prenylamine, another type of calmodulin antagonist that binds to CaM at sites different from the W-7 binding sites, did not inhibit the poly(L-aspartic acid)-induced activation of $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase. These results imply that poly(L-aspartic acid) is a calcium-independent activator of $Ca^{2+}$-dependent phosphodiesterase and that aspartic acids in the CaM molecule may play an important role in the activation of $Ca^{2+}$-PDE.

An extensive body of evidence supports the premise that $Ca^{2+}$–calmodulin is an intracellular regulator of diverse physiological processes. Calmodulin (CaM)[1] binds 4 mol of calcium and is thought to mediate the $Ca^{2+}$-dependent regulation of a large number of enzyme activities (Klee & Vanaman, 1982). Quantitative kinetic models are now available for calcium ion and CaM interactions with the $Ca^{2+}$–CaM-dependent phosphodiesterase (PDE) (Cox et al., 1981), and the mode of action of CaM with respect to the phosphodiesterase has been studied extensively. Using various hydrophobic fluorescent probes, it was found that when $Ca^{2+}$ binds to CaM, it induces a conformational change that exposes hydrophobic regions that may be responsible for the activation of enzymes (LaPorte et al., 1980; Tanaka & Hidaka, 1980). However, the molecular mechanism by which amino acids are primarily responsible for the activation remains obscure. On the other hand, alternate activators of the CaM-dependent phosphodiesterase such as phospholipids (Wolff & Brostrom, 1976), fatty acids (Hidaka et al., 1978a), gangliosides (Davis & Daly, 1980), and quinazolinesulfonamide derivatives (Tanaka et al., 1983) have been demonstrated. As these activators do not contain amino acid residues and most are insoluble in water, it is most difficult to speculate which amino acids in the CaM molecule are involved in the enzyme activation. To elucidate CaM–enzyme interactions and to characterize $Ca^{2+}$-dependent phosphodiesterase, we investigated the effects of various homopoly(amino acids) on the phosphodiesterase activity. We now report that poly(L-aspartic acid) is an activator of $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase, and the mechanism of activation by poly(aspartic acid) is discoursed in comparison with that of the $Ca^{2+}$–CaM complex.

## Experimental Procedures

*Materials.* Poly(L-aspartic acids) were purchased from Sigma Chemical Co. ($M_r$ 26 000) or Vega Biochemicals ($M_r$ 25 000–50 000). Poly(L-glutamic acid) ($M_r$ 30 600), poly(L-arginine) ($M_r$ 13 900), and poly(L-lysine) ($M_r$ 144 000) were from Miles Laboratories, Inc. Poly(L-proline) ($M_r$ 12 000) was from Sigma Chemical Co. *N*-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) and its derivatives were synthesized by the method of Hidaka et al. (1978b). Chlorpromazine (CPZ) and trifluoperazine (TFP) were donated by Yoshitomi Pharmaceutical Industries, Ltd. Prenylamine was supplied by Hoechst Japan, Ltd. *trans*-4-(Guanidinomethyl)cyclohexanecarboxylic acid *p-tert*-butylphenyl ester (GMCHA) was a kind gift from Professor K. Endo, School of Pharmacy, Tokushima University of Arts and Science. 3-(2-Benzothiazolyl)-4,5-dimethoxy-*N*-[3-(4-phenylpiperidinyl)propyl]benzenesulfonamide (HT-74) was donated from Takeda Chemical Industries, Ltd. [$^3$H]Guanosine cyclic 3′,5′-monophosphate, [$^3$H]adenosine cyclic 3′,5′-monophosphate, and adenosine 5′-[$\gamma$-$^{32}$P]triphosphate were from Amersham International, Ltd. All other chemicals were of reagent grade or better. Calmodulin (CaM) was purified from bovine brain by the method described by Yazawa et al. (1980). CaM-deficient $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase was purified by the method of LaPorte et al. (1979). Human blood platelets contain three distinct forms of cyclic nucleotide phosphodiesterase. Sonicated extracts of human blood platelets chromatographed on DEAE-cellulose yielded three cyclic nucleotide phosphodiesterase fractions designated as FI, FII, and FIII. FI was a specific cGMP phosphodiesterase at a low substrate concentration. FIII hydrolyzes cAMP faster than cGMP and with a high affinity. FI and FIII were used for this experiment, as cGMP and cAMP phosphodiesterases, respectively (Hidaka & Asano, 1976a).

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[1] Abbreviations: CaM, calmodulin; W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide; PDE, phosphodiesterase; CPZ, chlorpromazine; TFP, trifluoperazine; GMCHA, *trans*-4-(guanidinomethyl)cyclohexanecarboxylic acid *p-tert*-butylphenyl ester; HT-74, 3-(2-benzothiazolyl)-4,5-dimethoxy-*N*-[3-(4-phenylpiperidinyl)propyl]-benzenesulfonamide; EGTA, ethylene glycol bis($\beta$-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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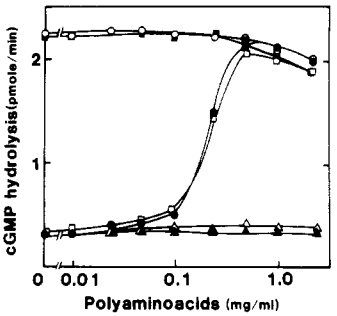


FIGURE 1: Effect of homopoly(amino acids) on $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase. Phosphodiesterase was assayed as described under Experimental Procedures in the presence of poly(L-aspartic acid) with $Ca^{2+}$–CaM (○) or $Ca^{2+}$ alone (□), poly(L-glutamic acid) (△), poly(L-proline) (▲), or poly(L-aspartic acid) (●) with 1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), or trypsinized phosphodiesterase with poly(L-aspartic acid) and 1 mM EGTA (■).

Myosin light chain kinase was purified from chicken gizzard by the method of Adelstein & Klee (1981). Myosin light chain of chicken gizzard, used as a substrate for the kinase assay, was prepared by the procedure of Perrie & Perry (1970). The light chain was separated from CaM by DEAE-cellulose chromatography (Adelstein et al., 1978).

*Methods.* Cyclic nucleotide phosphodiesterase activity was measured as previously described (Hidaka & Asano, 1976b). The reaction mixture contained 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 0.4 μM [$^3$H]guanosine cyclic monophosphate (100000 cpm) or 0.4 μM [$^3$H]adenosine cyclic monophosphate (100000 cpm), 1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) or 0.1 mM $CaCl_2$ and 400 ng/mL CaM, and the phosphodiesterase preparation, in a total volume of 0.5 mL. The reaction was initiated by addition of the substrate. CaM was assayed for its ability to stimulate a fixed amount of CaM-deficient phosphodiesterase, under standard conditions. One unit of CaM was defined as the amount activating 50% of a maximum of the phosphodiesterase attainable under standard experimental conditions and was equivalent to 10 ng of protein (Hidaka et al., 1980). Myosin light chain kinase activity was assayed, as previously described (Takana et al., 1980). Unless otherwise noted, 0.1 μM CaM was used.

## RESULTS

*Activation of PDE by Poly(aspartic acid).* As shown in Figure 1, $Ca^{2+}$-independent activation of $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase was observed only with poly(L-aspartic acid) and not with poly(L-glutamic acid), poly(L-arginine), poly(L-lysine), and poly(L-proline) (Figure 1). Poly(L-aspartic acid) activated $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase to the same extent in the presence or absence of calcium ion (Figure 1). The activation by poly(aspartic acid) was comparable to that obtained with the $Ca^{2+}$–calmodulin (CaM) complex. Poly(L-aspartic acid) produced no further stimulation of the $Ca^{2+}$-dependent phosphodiesterase, in the presence of the $Ca^{2+}$–CaM complex, and had a slight inhibitory effect on the CaM-activated enzyme activity above 1 mg/mL (Figure 1). Poly(L-glutamic acid) and poly(L-proline) did not activate $Ca^{2+}$-dependent phosphodiesterase in the presence or absence of calcium ion and also had a slight inhibitory effect on the enzyme activity with $Ca^{2+}$–CaM complex above 1 mg/mL. Poly(L-arginine) and poly(L-lysine) were potent inhibitors of CaM as previously reported (Itano et al., 1980).

The effect of poly(L-aspartic acid) on the cGMP concentration dependence of the enzyme was determined at a saturating concentration (0.5 mg/mL) of poly(L-aspartic acid). The poly(L-aspartic acid)-dependent phosphodiesterase activity exhibited an apparent $K_m$ of 2.7 μM for cGMP and a $V_{max}$ of 12 μmol min$^{-1}$ mg$^{-1}$ (Table I). This homopoly(amino acid) provided an apparent increase in $V_{max}$ of the phosphodiesterase associated with a decrease in the apparent $K_m$ for the substrate. The kinetic constants of the poly(L-aspartic acid)-activated enzyme were similar to those characteristics of the $Ca^{2+}$–CaM-dependent phosphodiesterase activity (Table I). However, the apparent $K_m$ of the enzyme activated by poly(L-aspartic acid) (1 mg/mL) for $Mg^{2+}$ was higher than that by the $Ca^{2+}$–CaM complex.

Table I: Effect of Poly(L-aspartic acid) on $Ca^{2+}$-Dependent Cyclic Nucleotide Phosphodiesterase[a]

| agent | apparent $K_m$ (μM) | $V_{max}$ (μmol min$^{-1}$ mg$^{-1}$) |
|---|---|---|
| none | 14.5 | 9.8 |
| poly(L-aspartic acid) | 2.7 | 12.0 |
| $Ca^{2+}$–CaM | 3.4 | 14.3 |

[a] Phosphodiesterase activity was measured with and without the addition of saturating concentrations of $Ca^{2+}$ (0.1 mM) and CaM (0.1 μM) and poly(L-aspartic acid) (0.5 mg/mL), as described under Experimental Procedure, with cGMP as the substrate. The $K_m$ and $V_{max}$ values were obtained from Lineweaver–Burk plots of the increments of activity in the presence of the added agents.

Multiple forms of cyclic nucleotide phosphodiesterase have been detected in mammalian tissues (Wells & Hardman, 1977). Three distinct cyclic 3',5'-nucleotide phosphodiesterase activity peaks (FI, FII, FIII) on DEAE-cellulose chromatography have also been detected in the case of the human heart, lung, liver, kidney, platelets, aorta, and cerebrum (Hidaka & Asano, 1976a; Hidaka et al., 1977, 1978a). FI and FIII phosphodiesterases from human platelets (Hidaka & Asano, 1976a) were used as cGMP and cAMP phosphodiesterases, respectively. Investigations undertaken to examine the effects of poly(L-aspartic acid) on three types of phosphodiesterase indicated that the poly(amino acid) is a selective activator of $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase (Figure 1), but not of cAMP (FIII) or cGMP (FI) phosphodiesterase.

Limited proteolysis of $Ca^{2+}$-dependent phosphodiesterase concomitantly activates the enzyme yet desensitizes it to further stimulation by CaM (Cheung, 1971). Similarly, the trypsin-treated $Ca^{2+}$-dependent phosphodiesterase was no longer sensitive to activation by poly(L-aspartic acid) (Figure 1). Poly(L-aspartic acid) and other poly(amino acids) failed to stimulate significantly myosin light chain kinase from smooth muscle, up to 2 mg/mL.

*Inhibitors of the Activation. N*-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) and other naphthalenesulfonamide derivatives, chlorpromazine (CPZ), trifluoperazine (TFP), prenylamine, GMCHA, and HT-74, were found to be CaM antagonists (Levin & Weiss, 1979; Hidaka et al., 1980), and the binding sites on calmodulin of prenylamine, GMCHA, and HT-74 were different from those of W-7, CPZ, and TFP (Hidaka et al., 1980). W-7, CPZ, and TFP also inhibited selectively poly(L-aspartic acid)-induced activation of $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase, as shown in Figure 2 and Table II. The addition of 0.5 mg/mL poly(L-aspartic acid) to the phosphodiesterase increased the enzymatic activity approximately 9-fold. Increasing the concentration of W-7, CPZ, and TFP progressively inhibited the activation of the phosphodiesterase. However, these compounds are less potent in inhibition of unactivated $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase in the absence of $Ca^{2+}$–CaM and poly(aspartic acid). Kinetic analysis of W-7-induced inhibition

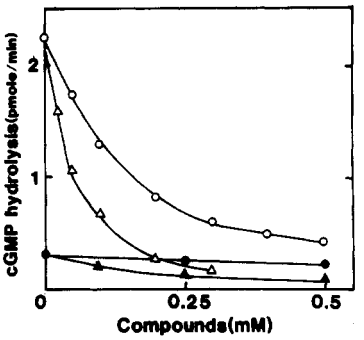


FIGURE 2: Effect of calmodulin antagonists on the activation of $Ca^{2+}$-dependent phosphodiesterase by poly(L-aspartic acid) (open symbols) and the basal activity without $Ca^{2+}$–CaM (closed symbols). Phosphodiesterase activity was measured at a series of concentrations of W-7 (○, ●) and chlorpromazine (△, ▲).

Table II: Effect of Calmodulin Antagonists on Poly(L-aspartic acid)-Induced Activation of $Ca^{2+}$-Dependent Cyclic Nucleotide Phosphodiesterase

| compound | $IC_{50}$[a] poly(aspartic acid) activated | $Ca^{2+}$–calmodulin activated | unactivated |
|---|---|---|---|
| W-7[b] | 100 | 26 | 1200 |
| W-5[c] | 300 | 240 | 3100 |
| W-9[d] | 54 | 14 | 820 |
| W-6[e] | 380 | 130 | 4000 |
| CPZ[f] | 38 | 16 | 130 |
| TFP[g] | 90 | 10 | 200 |
| prenylamine | 640 | 18 | 600 |
| GMCHA[h] | 500 | 8 | 350 |
| HT-74[i] | 200 | 1.8 | 200 |

[a] The concentration of a drug producing 50% inhibition of phosphodiesterase activation in the presence of 0.5 mg/mL poly(aspartic acid) and 400 ng/mL CaM is defined as $IC_{50}$ [poly(aspartic acid) activated] and $IC_{50}$ ($Ca^{2+}$–CaM activated), respectively. [b] *N*-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide. [c] *N*-(6-Aminohexyl)-1-naphthalenesulfonamide. [d] *N*-(6-Aminohexyl)-5-chloro-2-naphthalenesulfonamide. [e] *N*-(6-Aminohexyl)-2-naphthalenesulfonamide. [f] Chlorpromazine. [g] Trifluoperazine. [h] *trans*-4-(Guanidinomethyl)cyclohexanecarboxylic acid *p-tert*-butylphenyl ester. [i] 3-(2-Benzothiazolyl)-4,5-dimethoxy-*N*-[3-(4-phenylpiperidinyl)propyl]benzenesulfonamide.

of activation of phosphodiesterase by poly(L-aspartic acid) revealed that this agent inhibited the activity in a competitive fashion, and the $K_i$ value was 0.19 mM (Figure 3). The concentrations of W-7, CPZ, and TFP that inhibited the poly(L-aspartic acid)-induced activation of phosphodiesterase by 50% ($IC_{50}$ [poly(aspartic acid) activated]) were 100, 38 and 90 μM, respectively (Table II). These values are between those that inhibited the CaM-induced activated phosphodiesterase [$IC_{50}$ (CaM activated)] and the phosphodiesterase, in the absence of $Ca^{2+}$–CaM complex [$IC_{50}$ (unactivated)] (Table II). Moreover, the systematically synthesized CaM antagonists, naphthalenesulfonamide derivatives, were examined with regard to their ability to inhibit phosphodiesterase in the presence and absence of $Ca^{2+}$–CaM or poly(L-aspartic acid). We reported that the actions of W-7 as a CaM antagonist depended on the chlorination of the naphthalene ring (Hidaka et al., 1981; Tanaka et al., 1982). With regard to inhibition of the poly(aspartic acid)-induced activation, a structure–activity relationship, with or without chlorine, was similar to that with CaM antagonists, as shown in Table II.

On the other hand, prenylamine, GMCHA, and HT-74 (Hidaka et al., 1980), which have binding sites on calmodulin different from W-7, were found to be weak inhibitors of the phosphodiesterase activated by poly(L-aspartic acid). The concentrations of prenylamine, GMCHA, and HT-74 that

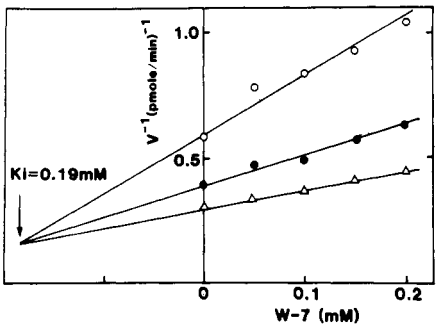


FIGURE 3: Kinetic analysis of W-7-induced inhibition of activation of $Ca^{2+}$-dependent phosphodiesterase by poly(L-aspartic acid) was carried out by using Dixon plots (Dixon, 1953). $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase activity was measured as described under Experimental Procedures with poly(L-aspartic acid): 0.2 (○), 0.4 (●), or 0.6 mg/mL (△).

inhibited the poly(L-aspartic acid)-induced activation of phosphodiesterase by 50% ($IC_{50}$ [poly(aspartic acid) activated]) were 640, 500, and 200 μm (Table II). These values are higher than the $IC_{50}$ (CaM activated) values and similar to $IC_{50}$ (unactivated) values of these compounds, respectively (Table II).

## DISCUSSION

We found that poly(L-aspartic acid) was a $Ca^{2+}$-independent activator of $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase. Total activity, as measured with saturating $Ca^{2+}$–CaM, was little changed, at concentrations below 1 mg/mL (Figure 1), thereby indicating that poly(L-aspartic acid) and $Ca^{2+}$–CaM competed for activation of the enzyme. Poly(L-aspartic acid) decreased in the $K_m$ for the substrate of $Ca^{2+}$-dependent phosphodiesterase and was associated with an increase in $V_{max}$, such being similar to the $Ca^{2+}$–CaM complex in its effect on the kinetic constants of the enzyme. Moreover, calmodulin antagonists such as W-7, CPZ, and TFP, which bind to the $Ca^{2+}$–CaM complex at the same sites (Hidaka et al., 1980), can inhibit the poly(L-aspartic acid)-induced activation of $Ca^{2+}$-dependent phosphodiesterase selectively. These results indicate that poly(L-aspartic acid) and the $Ca^{2+}$–CaM complex share significant common features as activators of phosphodiesterase. This poly(amino acid) appears to have the same structure or property as the active site of CaM, since CaM antagonists seem to competitively inhibit poly(aspartic acid)-induced activation of $Ca^{2+}$-PDE. Moreover, similar structure–activity relationships of naphthalenesulfonamides were also observed with regard to the inhibition of both poly(aspartic acid)-induced and $Ca^{2+}$–CaM-induced activation of the enzyme, thereby suggesting that W-7 and its derivatives, CPZ and TFP, may function by binding to poly(L-aspartic acid). Actually, we confirmed that [$^3$H]W-7 could bind to poly(L-aspartic acid), as seen with the gel filtration method. All these results suggest that poly(L-aspartic acid) may have physical properties in common with those of the $Ca^{2+}$–CaM complex. However, other types of CaM antagonists such as prenylamine, GMCHA, and HT-74, which have different binding sites on the CaM molecule from those of naphthalenesulfonamides and phenothiazines (Hidaka et al., 1980), failed to inhibit the poly(aspartic acid)-induced activation of $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase. Therefore, poly(L-aspartic acid) may not contain the binding domain of these drugs on CaM. On the other hand, the activation of $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase by other alternative activators such as phospholipids (Wolff & Brostrom, 1976), fatty acids (Hidaka et al., 1978a), and quinazolinesulfonamide derivatives (Tanaka et al., 1983)

was inhibited by prenylamine as well as by naphthalenesulfonamides and phenothiazines. In other words, poly(L-aspartic acid) is an incomplete $Ca^{2+}$–CaM complex, with a W-7 binding and PDE activating domain but not a prenylamine binding one.

It has been postulated that $Ca^{2+}$ binding exposes hydrophobic regions on the surface of CaM and which are recognized by CaM-dependent enzymes and CaM antagonists such as W-7 and phenothiazines (LaPorte et al., 1980; Tanaka & Hidaka, 1980). Common to all compounds is that they are not only hydrophobic but also positively charged. The major forces contributing to the binding of these CaM antagonists to CaM may be hydrophobic interactions, and the binding specificity is most likely controlled by electrostatic interactions where the positive charges on these compounds are properly positioned, with respect to the hydrophobic center. Moreover, chemical modification of carboxyl groups of CaM resulted in loss of the biological activity (Walsh & Stevens, 1977). In the present work, we demonstrated that aspartic acids in the CaM molecule play an important role in the activation of $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase. Although it has been reported that the CD spectrum of poly(L-aspartic acid) differs from the spectra of other ionizable homopolypeptides such as poly(L-glutamic acid) in the ionized state (Saudek et al., 1982), it is not clear why poly(L-glutamic acid) did not stimulate $Ca^{2+}$-dependent phosphodiesterase. However, as poly(L-aspartic acid) is soluble in contrast with other alternate activators, it seems to be more useful for analysis of the interaction between $Ca^{2+}$-dependent phosphodiesterase and this activator. Studies are under way to determine precisely the differences in conformation of poly(L-aspartic acid) and poly(L-glutamic acid). The molecular mechanism of poly(aspartic acid)-induced activation and the role of aspartic acid residues in CaM function probably will thus be clarified.

Poly(L-aspartic acid) is similar to other alternate stimulators such as acidic phospholipids and synthetic quinazolinesulfonamides with regard to the incomplete activation of myosin light chain kinase. In the case of $Ca^{2+}$,$Mg^{2+}$-ATPase in human erythrocytes, poly(L-aspartic acid) was shown to increase the $Ca^{2+}$ sensitivity but not the maximum velocity of the enzyme (Minocherhomjee et al., 1982). These results suggest that all these alternate activators may possess physical properties required for activation of phosphodiesterase but not of the myosin light chain kinase. Thus, different domains of CaM may be responsible for activation of each enzyme (Newton et al., 1984).

In summary, the data presented in this study indicate that poly(L-aspartic acid) is similar to the $Ca^{2+}$–CaM complex in activation of $Ca^{2+}$-dependent cyclic nucleotide phosphodiesterase and in inhibition of this activation by some CaM antagonists such as W-7 and phenothiazines. However, other types of CaM antagonists such as prenylamine, GMCHA, and HT-74, which have different binding sites on CaM, did not inhibit the poly(L-aspartic acid)-induced activation of $Ca^{2+}$-PDE. These results suggest that aspartic acid residues in the CaM molecule may play an important role in the activation of $Ca^{2+}$-PDE. Poly(L-aspartic acid) possesses physical properties seen in the incomplete $Ca^{2+}$–CaM complex and should prove to be a useful tool for the study of CaM and $Ca^{2+}$-dependent phosphodiesterase, in vitro.

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**Registry No.** W-5, 79458-81-4; W-6, 84215-11-2; W-7, 65595-90-6; W-9, 84215-10-1; CPZ, 50-53-3; TFP, 117-89-5; GMCHA, 81907-78-0; HT-74, 97467-29-3; prenylamine, 390-64-7; cGMP phosphodiesterase, 9068-52-4; cAMP phosphodiesterase, 9036-21-9; poly(L-aspartic acid), 25608-40-6; poly(L-aspartic acid), SRU, 26063-13-8.

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