Hydrophobic properties of *Tetrahymena* calmodulin related to the phosphodiesterase activity

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We have examined hydrophobic properties of *Tetrahymena* CaM using the uncharged probe, *n*-phenyl-1-naphthylamine (NPN) fluorescence. The maximal fluorescence intensity of *Tetrahymena* calmodulin (CaM) is <1/12 of that of the bovine brain CaM. In the phosphodiesterase activation, the potency of *Tetrahymena* CaM, which was represented by reciprocals of the quantity of CaM required for half-maximal activation of enzyme was 22.7% respectively, of that of the bovine brain CaM. Here, *Tetrahymena* CaM had less hydrophobic groups exposed in the presence of Ca^{2+}. Then Ca^{2+}-CaM dependent enzymes require much amount of *Tetrahymena* CaM, comparing with the bovine brain CaM.

Tetrahymena pyriformis *Calmodulin* *Ca^{2+}–CaM-dependent phosphodiesterase*
N-*Phenyl-1-naphthylamine* *Hydrophobicity* *Calcium*

1. INTRODUCTION

In [1] it was noted that the activity of membrane-bound guanylate cyclase from *Tetrahymena pyriformis* was activated by an endogenous protein, in the presence of Ca^{2+}. This activator protein proved to be a Ca^{2+}-binding protein of acidic and thermostable nature and attention was given to the possibility that this compound was calmodulin (CaM) [1]. As this protein activated Ca^{2+}–CaM-dependent phosphodiesterase to a lesser extent than did bovine brain CaM, the question followed as to why Ca^{2+}–CaM-dependent phosphodiesterase requires a larger amount of *Tetrahymena* CaM, compared with bovine brain CaM.

A variety of techniques used to demonstrate Ca^{2+}-induced conformational change in CaM include optical rotatory dispersion [2], circular dichroism [3], differential sensitivity to proteolysis [4], differential sensitivity to chemical modification [2], absorption spectroscopy [5] and enhancement of tyrosine fluorescence [6]. Finally, the functional significance of Ca^{2+}-induced conformational change was explained on the basis that the exposed hydrophobic groups of CaM were related to the activation of Ca^{2+}–CaM-dependent enzymes, determined using several hydrophobic probes [7,8].

We now report that *Tetrahymena* CaM has fewer hydrophobic groups exposed in the presence of Ca^{2+}, thus, Ca^{2+}–CaM-dependent enzymes require a larger amount of *Tetrahymena* CaM compared with bovine brain CaM.

2. MATERIALS AND METHODS

2.1. *Protein*

Calmodulin (CaM) of bovine brain and *Tetrahymena* was purified as in [1,9]. The preparations were assessed to be homogenous by sodium dodecylsulfate–polyacrylamide gel electrophoresis. CaM-deficient Ca^{2+}-dependent 3′:5′-cyclic nucleotide phosphodiesterase from bovine brain was partially prepared as in [5].

Abbreviations: CaM, calmodulin; NPN, *N*-phenyl-1-naphthylamine; EGTA, ethylene glycol bis (β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid

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2.2. *Fluorescence measurements*

Fluorescence measurements were made at room temperature (22°C) using microcuvettes with an Aminco-Bowman fluorescence spectrophotometer with a high stability xenon lamp scanning from 360–600 nm. Total dilution never exceeded 5% and relative fluorescence values were uniformly corrected for dilution. *N*-phenyl-1-naphthylamine (NPN) was dissolved in 100% ethanol, and concentration of ethanol in the samples undergoing measurement was never >0.5%.

2.3. *Activity of bovine brain cyclic nucleotide phosphodiesterase*

Activity of bovine brain cyclic nucleotide phosphodiesterase using cyclic GMP as substrate was measured as in [10].

2.4. *Protein determination*

Protein was determined as in [11], with purified CaM as a standard.

3. RESULTS

3.1. *Phosphodiesterase activity of* Tetrahymena *CaM and bovine brain CaM*

Tetrahymena and bovine brain CaM's activated CaM-deficient brain phosphodiesterase in the presence of Ca^{2+} (fig.1). Although the extent of the maximal activation of phosphodiesterase produced by *Tetrahymena* CaM was comparable to that of bovine brain CaM, the amount of CaM required for half-maximal activation of the enzyme differed. In the activation by phosphodiesterase, the potency of *Tetrahymena* CaM represented by reciprocals of the quantity of CaM required for half-maximal activation of enzyme was 22.7% bovine brain CaM. The concentrations of Ca^{2+} required for half-maximal activation of enzyme were 2 and 4 μM with bovine brain *tetrahymena* CaMs (not shown) [1].

3.2. *Interaction between* n*-phenyl-1-napthylamine (NPN) and CaM*

Fig.2 shows that the bovine brain CaM and *Tetrahymena* CaM interact with NPN in the presence of 20 μM Ca^{2+}. The fluorescence at 420 nm of NPN in the presence of two proteins was negligible with $\geqslant 1$ μM Ca^{2+} in the reaction mixture. Ca^{2+} increased the quantum yield and a 'blue shift' occurred in the emission peaks. In the absence of CaM, Ca^{2+} had no effect on the fluorescence of NPN. When the effect of Ca^{2+} concentration on the interaction was studied using the Ca^{2+}-EGTA buffer system, the NPN fluorescence was maximal with 20 μM Ca^{2+} in the bovine brain CaM, and with 5 μM Ca^{2+} it was maximal with 20 μM Ca^{2+}

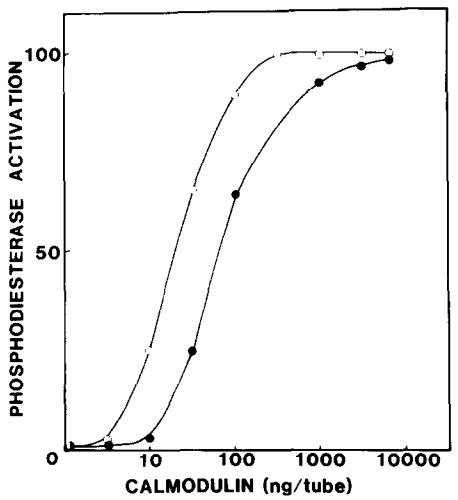

Fig.1. Effect of the bovine brain CaM (—○—) and the *Tetrahymena* CaM (—●—) on the activity of Ca^{2+}–CaM-dependent phosphodiesterase. The result is expressed as a % of maximal activation plotted against the amount of CaMs (ng/tube) added.

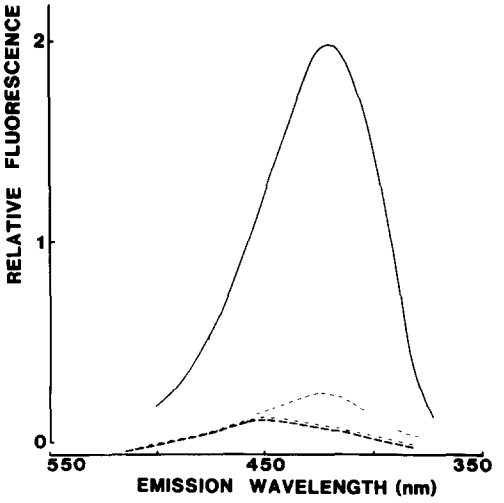

Fig.2. The bovine brain CaM and the *Tetrahymena* CaM interact with NPN in the presence of 20 μM Ca^{2+} (——) (----), and 1 mM EGTA (– – –) (–·–·–). Solutions contained 50 mM Tris–HCl (pH 7.6) 10 μM CaM and 10 μM NPN.

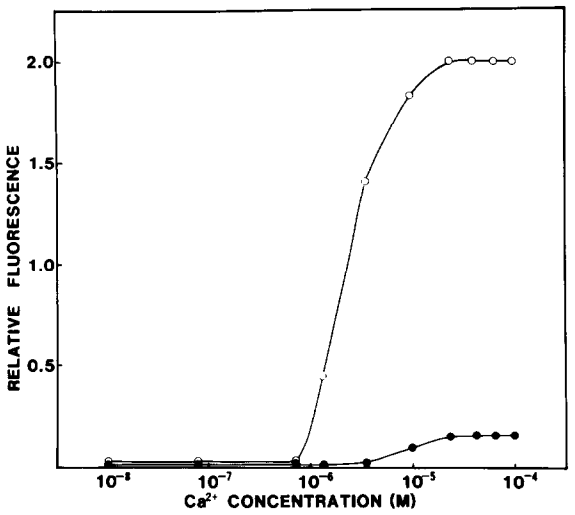

Fig.3. NPN fluorescence increased as a function of Ca^{2+} concentration in the presence of the bovine brain CaM (—○—) and the *Tetrahymena* CaM (—●—). Solutions contained 50 mM Tris–HCl (pH 7.6) 10 μM CaM, 10 μM NPN and Ca^{2+}-EGTA buffer as indicated.

in *Tetrahymena* CaM. The maximum fluorescence intensity of *Tetrahymena* CaM was <1/12 that of the bovine brain CaM (fig.2). Addition of excess EGTA of the NPN–CaM complex or dialysis of the complex reduced the fluorescence significantly and re-addition of Ca^{2+} and NPN increased the fluorescence, suggesting the reversibility of interaction between NPN and the Ca^{2+}–CaM complex. In the presence of μM levels of Ca^{2+}, a hydrophobic site of CaM becomes accessible to NPN. Our findings show that *Tetrahymena* CaM has fewer hydrophobic groups exposed in the presence of Ca^{2+}, compared with bovine brain CaM. The Ca^{2+}–CaM-dependent phosphodiesterase requires larger amounts of *Tetrahymena* CaM than does bovine brain CaM.

4. DISCUSSION

Ca^{2+}–CaM-dependent enzymes such as Ca^{2+}-dependent cyclic nucleotide phosphodiesterase [12, 13], myosin light-chain kinase [14], and the erythocyte Ca^{2+}-ATPase [15] can be activated by the addition of acidic phospholipids or of fatty acids. These phenomena suggested to us that Ca^{2+}–CaM interacts hydrophobically with Ca^{2+}–CaM-dependent enzymes.

A number of amphiphilic cationic antipsychotic drugs bind to CaM and inhibit CaM stimulation of the Ca^{2+}-sensitive phosphodiesterase [16]. Although some antipsychotic drugs exhibit stereospecificity for certain in vitro and in vivo effects, binding to CaM, and inhibition of CaM stimulation of phosphodiesterase were not nearly so stereospecific [17]. Recently, we found that change in the affinity of antagonists for CaM correlates well with change in the hydrophobicity, determined by using 3 series of naphthalenesulfonamide derivatives [18].

The fluorescence intensity of NPN increased dramatically in the presence of Ca^{2+} and calmodulin. With this ligand, there was a significant shift in the emission spectrum to a shorter wave-length. In contrast, CaM had only a slight effect on the emission spectra of this probe, in the presence of EGTA, and Ca^{2+} alone had no effect on the fluorescence of this ligand. This spectral change is consistent with findings in case of removal of the fluorescent dye from an aqueous environment to hydrophobic binding sites on the protein [19].

These results show that the activities of *Tetrahymena* and bovine brain CaMs for Ca^{2+}–CaM-dependent phosphodiesterase correlate well with amount of the exposed hydrophobic groups of CaMs in the presence of Ca^{2+}.

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