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# THE DIFFERENTIAL STIMULATION OF BRAIN AND HEART CYCLIC-AMP PHOSPHODIESTERASE BY ONCOMODULIN

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$Ca^{2+}$/ calmodulin dependent cyclic nucleotide phosphodiesterase, from the bovine heart and brain, purified by monoclonal antibody chromatography were tested with respect to activation by oncomodulin. The heart and brain enzymes which have previously been shown to have slightly different electrophoretic mobilites (1), were found to also differ in the oncomodulin dose-dependent activation of cAMP hydrolysis. Oncomodulin was shown to activate the heart enzyme to the same extent as calmodulin. However, this study indicates that the heart phosphodiesterase has approximately 25-fold higher affinity for oncomodulin than the brain enzyme. The oncomodulin concentration required for the half-maximal activation of the heart phosphodiesterase was estimated to be $2 \times 10^{-7}$M. In addition, the possibility of the observed activation by oncomodulin being due to calmodulin contamination can be ruled out as the oncomodulin activation profiles were unaltered subsequent to chromatography on organomercurial agarose and the activation by oncomodulin could not be reversed by anti-calmodulin IgG. 

Oncomodulin is a 108 residue oncodevelopmental calcium-binding protein found in human and rodent placenta, and tumors of various tissues, but not in any normal fetal or adult organ (1-3). It's primary structure is that of a β-parvalbumin (4), but the molecule is different from that of the parvalbumins in having a calcium-specific conformational change (5,6).

In 1981, another difference from the parvalbumins was described, a calmodulin-like ability by oncomodulin to stimulate the hydrolysis of cyclic-AMP by rat heart phosphodiesterase (7). Oncomodulin is not as promiscuous as

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the 148 residue calmodulin, being unable to affect the activity of calcium-ATPase, or phosphorylase b kinase (1), or calcineurin phosphatase (8), myosin light chain kinase (9), microtubule assembly or caldesmon binding (10), or cerebrosidase (11) all of which are influenced by calmodulin. However, in 1984 in this journal, in a supposed repeat of the earlier work, Klee and Heppel failed even to find that oncomodulin stimulated cyclic-AMP phosphodiesterase, but had used bovine brain enzyme (12).

In reply to this detraction, we have re-examined these phenomena since it is well known that the phosphodiesterase enzymes from heart and brain are different (13).

## MATERIALS AND METHODS

The cyclic AMP phosphodiesterase from bovine heart and brain was purified using anti-phosphodiesterase monoclonal antibodies as immunoaffinity reagents (14). The enzyme preparations were homogenous by polyacrylamide gel electrophoresis showing one band at 59Kd for heart, and 61Kd for brain as previously described (13). Activity was measured as described by Sharma and Wang (15).

The oncomodulin was purified from rat Morris hepatoma (16). The material eluting from Sephadex G50 was further purified by HPLC (17) on a C-3 reverse column (RPSC, Beckman, 4.6 x 75 mm) equilibrated in 10 mM Tris HCl, 1 mM EGTA, pH 7.2, eluted with a gradient (1%/min) composed of 40% equilibration buffer: 60% n-propanol. The calmodulin was from rat testes or bovine brain (18), and the parvalbumin was from rat skeletal muscle (4). The anti-calmodulin IgG was purchased from Biomedical Technologies (Cambridge, Mass.)

Oncomodulin and calmodulin were also subjected to sulphydryl affinity chromatography on organomercurial agarose (19) which had a binding capacity of 0.6 mg bovine serum albumin/ml. The HPLC purified oncomodulin (145 μg) was dissolved in 0.5 ml of buffer A (20 mM Tris HCl. 150 mM KCl 0.1 mM EGTA pH 7.0) containing 10 mM β-mercaptoethanol. The protein sample was incubated at room temperature for 30 min, and stripped of β-mercaptoethanol by chromatography on Sephadex G-25. The reduced protein was then applied to a column of organo-mercurial agarose (2ml) pre-equilibrated with buffer A. Separate columns of derivatized agarose were used for the different proteins. These affinity columns were washed with a further 100 ml of equilibrating buffer. The absorbed protein was then eluted from the column with buffer A containing mM β-mercaptoethanol. The material eluted with the reducing agent was dialysed against buffer A containing no EGTA, but 0.1 mM $CaCl_2$, 10 mM β-mercaptoethanol.

## RESULTS AND DISCUSSION

The primary initial concern was to have the oncomodulin used for enzyme activity studies absolutely free of possible calmodulin contamination. It was possible by HPLC to separate, with baseline resolution, oncomodulin from calmodulin or parvalbumin (fig 1A), and to show that purified oncomodulin was free of calmodulin contamination (fig 1B). However additional assurance of

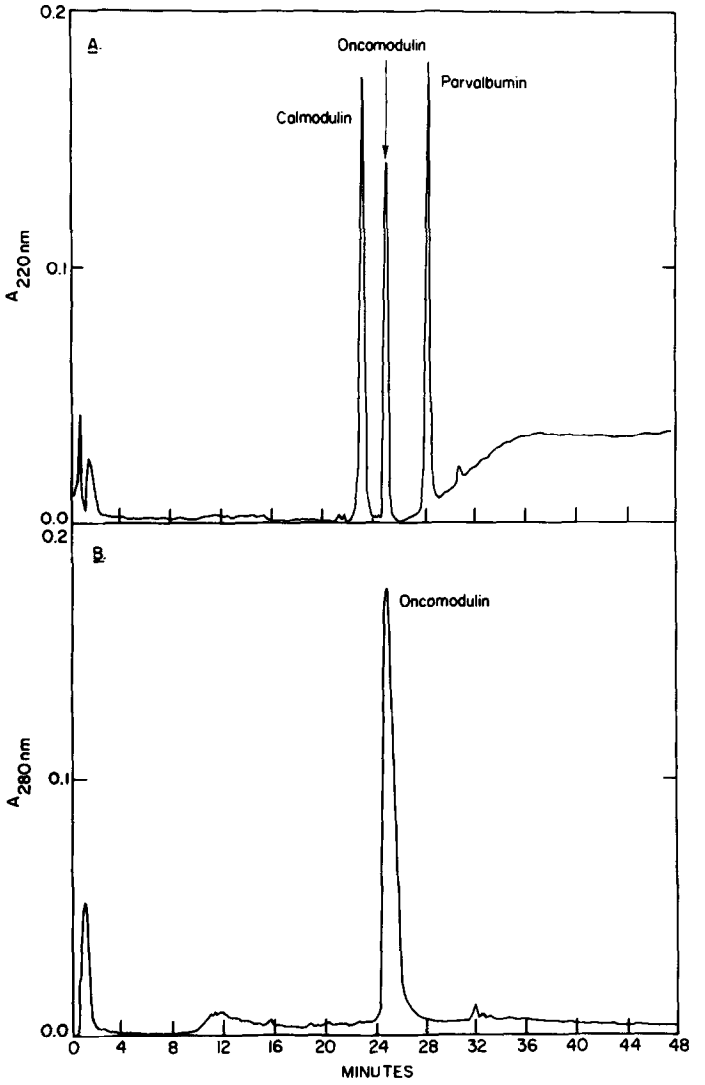
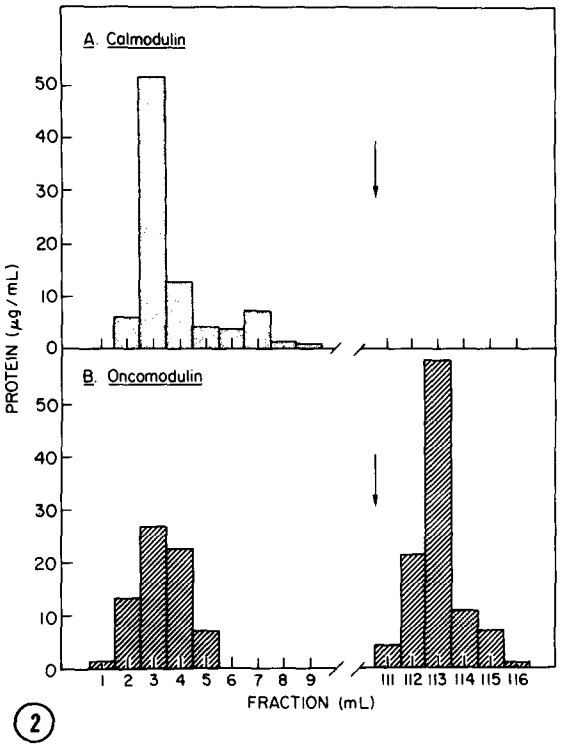



fig. 1. The reverse-phase HPLC purification of oncomodulin free of calmodulin contamination. A: Demonstration of base-line resolution in separation of calmodulin, oncomodulin and parvalbumin (20 μg of each protein). B: Preparative purification of oncomodulin (1 mg) showing no evidence for presence of calmodulin.

purity was achieved by taking advantage of the Cys-18 of oncomodulin (4,6) in use of organomercurial affinity chromatography (fig 2). Mammalian calmodulin lacks cysteine (20), and so passed through such an affinity resin (fig 2A). On the other hand, the majority of oncomodulin was retained, to be eluted with β-mercaptoethanol (fig 2B).

Both the HPLC pure, and the sulphydryl affinity purified oncomodulin preparations identically stimulated heart phosphodiesterase approximately five-



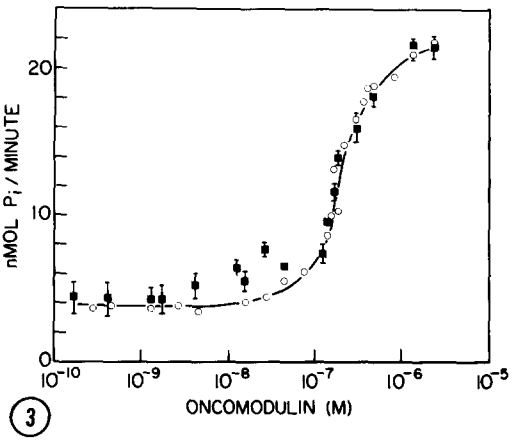




fig. 2. Use of sulphydryl affinity chromatography to obtain oncomodulin free of possible calmodulin contamination. A: Calmodulin was not retained and B: The thiol-reduced fraction of HPLC pure oncomodulin (fig 1B) was retained by the organomercurial resin. The arrow indicates the start of elution with β-mercaptoethanol.

fig. 3. Equal stimulation of bovine heart phosphodiesterase by oncomodulin from fig 1B ( ■ ) and fig 2B ( ○ ). Values given are mean ± SEM of at least three separate determinations.

fold (fig 3). The concentration of oncomodulin required for half-maximal activation of this bovine heart enzyme was estimated to be $2 \times 10^{-7}$M, in agreement with that originally found with the rat heart enzyme. The activation was calcium-dependent, being abolished by the chelator EGTA (not shown), as was seen previously with the rat enzyme (7).

A comparison of the stimulation of heart and brain phosphodiesterase by both calmodulin and oncomodulin is presented in fig 4. Both calcium-binding proteins stimulated the heart enzyme activity to the same extent (fig 4A). However the heart and brain enzymes had different apparent relative affinities for calmodulin, which was even more obvious with oncomodulin. The concentration of calmodulin required for half-maximal activation of heart phosphodies-

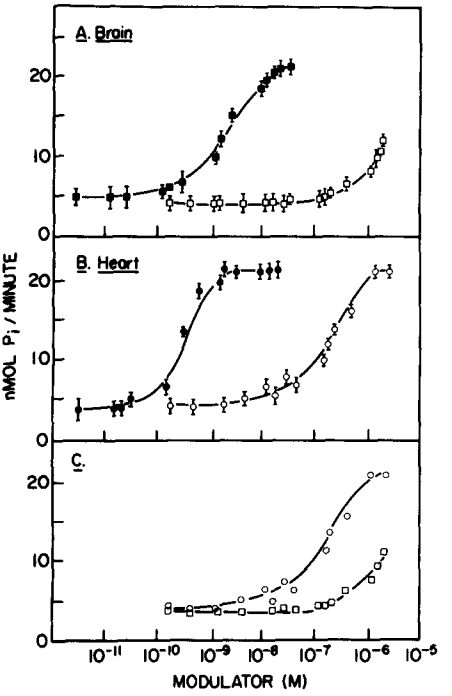



fig. 4. Differential stimulation of brain (A) and heart (B)phosphodiesterase by oncomodulin ( □ or ○ ), and calmodulin ( ■ or ● ). Values given are mean ± SEM of at least three separate determinations. C: Replot of A & B to demonstrate the greater sensitivity to oncomodulin of phosphodiesterase from heart compared to brain.

terase ($5 \times 10^{-10}$M) was about four fold lower than for the brain enzyme ($2 \times 10^{-9}$M). These differences were more dramatic for oncomodulin, where the heart enzyme had an apparent affinity ($2 \times 10^{-7}$M) twenty-five fold lower than that estimated for the brain enzyme (fig 4C). This was an additional demonstration that calmodulin contamination was not responsible for oncomodulin oncomodulin stimulation. In addition, anti-calmodulin IgG added to the reaction mixture was able to reduce the stimulation by calmodulin, but not by oncomodulin (Table 1). The stimulation of bovine heart phosphodiesterase was specific for oncomodulin, since another similarly sized calcium-binding protein, parvalbumin, with 55% identity of primary structure (4) was completely incapable of any stimulation of enzyme activity (not shown). This had already been noted with the rat heart enzyme (7).

Thus, the parvalbumin-like protein, oncomodulin, was able to stimulate bovine heart phosphodiesterase activity in a calcium-dependent manner, as well

TABLE 1 The Effect of Anti-CaM IgG on Calmodulin and Oncomodulin stimulated heart-PDE activity

| | v (nmol cAMP hydrolysed / min) | |
|---|---|---|
| | -Ig G | +Ig G |
| NO STIMULATOR | 4.8 (±.15) | - |
| Calmodulin ($5.5 \times 10^{-9}$M) | 15.4 (±.36) | 10.3 (±.50) |
| Oncomodulin($5.0 \times 10^{-7}$M) | 11.2 (±.50) | 11.0 (±.60) |

as calmodulin, albeit at higher concentrations, in a similar manner to the rat enzyme (7). The heart enzyme appeared to be different from the brain enzyme in sensitivity to oncomodulin, and to a lesser degree to calmodulin, which extends the electrophoretic differences previously reported (13). Finally the inability of Klee and Heppel (12) to reproduce the original reported modulator action of oncomodulin can now be seen as a failure to repeat the original experiments, and an unfortunate choice of alternate enzyme source.

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