Purification and characterization of the 27 000 Da calcium-binding protein of bovine brain

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A Ca^{2+} -binding protein named CAB-27 was purified from bovine brain 100 000 g supernatant. The protein has a molecular mass of 27 000 Da as determined by SDS/polyacrylamide-gel electrophoresis and 35 500 Da by sedimentation-coefficient and Stokes-radius analysis. The protein contains about 26% Glx and Asx and 13% basic residues. The acidic nature of the molecule is confirmed by its pI of 4.80. In the presence of 3 mM-MgCl₂ and 150 mM-KCl, CAB-27 binds 2.0 mol of Ca^{2+} /mol of protein, with an apparent K_d of 0.2 μM . Ca^{2+} binding is unaffected by prior incubation of the protein at 80 °C for 2 min. Brain contains about 130 mg of CAB-27/kg. Immunoblotting identified CAB-27 in several bovine tissues; it appears to be particularly rich in brain and kidney. In addition, CAB-27 is identified as an inhibitor of bovine pancreas phospholipase A₂ *in vitro*. The inhibitory activity of CAB-27 was 20-fold less potent than lipocortin. On the basis of the Ca^{2+} -binding properties, intracellular concentration and tissue distribution of this protein, we suggest that CAB-27 may be an important intracellular Ca^{2+} receptor.

INTRODUCTION

The development of techniques to allow quantification of changes in cytosolic free Ca^{2+} concentrations with varied stimuli has catalysed a resurgence of interest in the Ca^{2+} second-messenger system. Information obtained from intracellular Ca^{2+} indicators such as aequorin (Shimomura & Johnson, 1970) and quin2 (Tsien & Rink, 1980) has provided solid evidence for a second-messenger role of Ca^{2+} in many cellular processes, including fibroblast proliferation (Moolenaar *et al.*, 1984; Morris *et al.*, 1984), sea-urchin egg fertilization (Steinhardt *et al.*, 1977; Eisen *et al.*, 1984), muscle contraction (Harvey *et al.*, 1985) and secretion (Berridge, 1984). The cytosolic Ca^{2+} -binding proteins are the intracellular receptors for second-messenger Ca^{2+} .

In previous work (Waisman *et al.*, 1983b), the 100 000 g supernatant of bovine brain was chromatographed on DEAE-cellulose, and the resultant fractions were assayed for Ca^{2+} -binding activity by the Chelex competitive Ca^{2+} -binding assay (Waisman & Rasmussen, 1983), and for calmodulin by the cyclic AMP phosphodiesterase assay (Teo *et al.*, 1973). Since the calmodulin activity comprised only a small percentage of the total Ca^{2+} -binding activity, we suggested that bovine brain contained, in addition to calmodulin, many other Ca^{2+} -binding proteins. Gel-permeation-chromatographic analysis of the Ca^{2+} -binding activity peaks eluted from DEAE-cellulose suggested that bovine brain might contain previously uncharacterized Ca^{2+} -binding proteins (Waisman *et al.*, 1983b). The three peaks of Ca^{2+} -binding activity, resolved by DEAE-cellulose chromatography, have been subjected to further purification, and the Ca^{2+} -binding proteins responsible for the Ca^{2+} -binding activity peaks have been purified and identified (Waisman & Rasmussen, 1983). Peak I was resolved into CAB-27 (Waisman *et al.*, 1983a, 1985a), peak II yielded CAB-48 (Waisman *et al.*, 1985a) and

calcineurin (Klee & Krinks, 1978), and peak III resolved into calregulin (Waisman *et al.*, 1985a) and calmodulin (Klee *et al.*, 1980).

In the present paper a rapid large-scale purification procedure for CAB-27 is documented. In addition, CAB-27 is characterized as a monomeric acidic Ca^{2+} -binding protein, whose Ca^{2+} -binding properties and tissue concentration suggest that it may be an important cytosolic Ca^{2+} receptor. Analysis of the function of CAB-27 has identified this protein as a specific inhibitor of phospholipase A₂ activity *in vitro*.

EXPERIMENTAL

Materials

All chemicals were reagent grade unless specified. Deionized water was used throughout. Chelex-100 and hydroxyapatite were obtained from Bio-Rad. Di-isopropyl fluorophosphate, benzamidine, phenylmethanesulphonyl fluoride and pig pancreatic phospholipase A₂ were obtained from Sigma. DEAE-cellulose (DE-52) was obtained from Whatman. L- α -1-Palmitoyl-2-[¹⁴C]-oleoylphosphatidylcholine was purchased from Amersham. Calmodulin (bovine brain), S-100_{ββ} (bovine brain) and parvalbumin (rabbit skeletal muscle) were generously given by Dr. J. H. Wang (University of Calgary). Calregulin (Waisman *et al.*, 1985b) and CAB-48 (Waisman *et al.*, 1985a) were purified as detailed in those references.

DEAE-cellulose chromatography of bovine brain 100 000 g supernatant

Fresh bovine brain was obtained from a local slaughterhouse. Connective tissue was dissected away, and the tissue was rinsed thoroughly with ice-cold distilled water and frozen immediately at -20 °C. Frozen tissue (1 kg) was chopped, and then further

Abbreviations used: DTT, dithiothreitol; PAGE, polyacrylamide-gel electrophoresis.

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minced in a meat grinder. The mince was mixed with 3 litres of ice-cold buffer, containing 40 mM-Tris/HCl (pH 8.0), phenylmethanesulphonyl fluoride (0.5 mM), soya-bean trypsin inhibitor (5 mg/l), di-isopropyl fluorophosphate (1.0 mM), leupeptin (5 mg/l), pepstatin (5.0 mg/l), antipain (5.0 mg/l), chymostatin (5.0 mg/l), benzamidine (10.0 mM) and DTT (1.0 mM), and then homogenized in a Waring blender. The resultant extract was centrifuged at 20000 *g* for 30 min, and the supernatant was then centrifuged at 100000 *g* for 60 min. The supernatant was diluted into 5 vol. of 40 mM-Tris/HCl (pH 8.0)/1.0 mM-DTT, and 800 ml of packed DEAE-cellulose was then added. The mixture was stirred rapidly for 1 h, and then filtered through a coarse sintered-glass funnel. The resultant slurry was washed with 6 litres of 40 mM-Tris/HCl (pH 8.0) and poured into a 5.0 cm × 60 cm column. Protein was eluted with a linear gradient made from 2.2 litres each of 40 mM-Tris/HCl (pH 8.0)/1.0 mM-DTT and 40 mM-Tris/HCl (pH 8.0)/1.0 mM-DTT/0.45 M-NaCl. The resulting fractions were analysed for both Ca²⁺-binding activity (by using the Chelex-100 competitive Ca²⁺-binding assay; Waisman & Rasmussen, 1983) and calmodulin activity (Teo *et al.*, 1973). Three peaks (I–III) of Ca²⁺-binding activity were pooled, and subsequently the Ca²⁺-binding protein responsible for the Ca²⁺-binding activity peak I was subjected to further purification.

Purification of CAB-27 from peak I

The pooled peak was dialysed against buffer A (1.0 mM-sodium phosphate buffer, pH 6.8, 1 mM-DTT), and applied to a 5.0 cm × 30 cm column of hydroxyapatite previously equilibrated with buffer A. The column was washed with 2 column volumes of buffer A and eluted with 2 litres of buffer A containing a linear phosphate gradient (1.0–100 mM). Fractions (25 ml) were collected and analysed for Ca²⁺-binding activity by Chelex-100 assay. The Ca²⁺-binding activity peak was heated at 80 °C for 2 min, and the denatured protein was removed by centrifugation at 30000 *g* for 30 min. The supernatant was concentrated by ultrafiltration (PM 10; Amicon Corp.), dialysed against buffer B (40 mM-Mops, pH 7.1, 150 mM-NaCl and 1 mM-DTT), applied to a high-performance gel-permeation liquid-chromatography column (TSK-G3000SW; LKB), and eluted with buffer B at a flow rate of 30 ml/h. Fractions (3.0 ml) were collected and assayed for Ca²⁺-binding activity.

Ca²⁺ binding of purified CAB-27

This was determined by equilibrium dialysis. CAB-27 was first dialysed overnight against 1000 vol. of a solution containing 150 mM-KCl, 10 mM-Mops (pH 7.1), 3 mM-MgCl₂, 1.0 mM-DTT and 0.1 mM-EGTA, to remove bound Ca²⁺ from the protein. The dialysed protein was then used for equilibrium dialysis as follows. A 0.5 ml portion of protein was dialysed with shaking for 48 h at 4 °C against 100 ml of the same solution (see above) containing various amounts of CaCl₂ and ⁴⁵Ca²⁺ (5 μ Ci) to achieve the desired free Ca²⁺ concentration. The solutions outside and inside the dialysis tubing were removed, the *A*₂₇₈ was determined, and then the protein concentration calculated (*A*₂₇₈^{1%} = 5.72). Portions of these solutions were subjected to liquid-scintillation spectrometry. The association constants for metal and H⁺ binding to EGTA were based on values measured by Fabiato (1981).

Amino acid analysis

CAB-27 was dialysed overnight against two changes of 1000 vol. of 40 mM-NH₄HCO₃, and portions of protein solutions were transferred to hydrolysis tubes containing 0.1% phenol and 0.22% 2-mercaptoethanol; 10 nmol of norleucine was used as an internal standard, to correct for losses of protein. Hydrolysis was carried out at 110 °C for 24, 48 and 72 h. The calculated values were based on mean values from eight determinations. Performic acid oxidation was performed as described by Hirs (1967). Tryptophan determinations were performed as detailed by Simpson *et al.* (1976). Recoveries of tryptophan typically ranged between 90 and 95%.

Sedimentation-velocity experiments

These were carried out at 40000 rev./min at 18 °C (Yphantis, 1964). Data were corrected to the density and viscosity of water at 20 °C. The partial specific volume was determined (Cohn & Edsall, 1943). The absorption coefficient was estimated from protein concentration determined by amino acid analysis. The Stokes radius was determined on a calibrated TSK-G3000SW column. The molecular mass was determined from Stokes-radius and sedimentation-coefficient data (Siegel & Monty, 1966).

Other methods

Agarose-gel isoelectric focusing (Saravis *et al.*, 1979), ⁴⁵Ca²⁺ autoradiography (Maruyama *et al.*, 1984), Chelex competitive assay (Waisman & Rasmussen, 1983), protein determination (Bradford, 1976), calmodulin assay (Teo *et al.*, 1973), phospholipase A₂ assay (Khanna *et al.*, 1986) and SDS/PAGE (Laemmli, 1970) were performed as described in the references.

RESULTS AND DISCUSSION

Purification of bovine brain CAB-27

Fig. 1 shows the DEAE-cellulose profile of 100000 *g* supernatant of bovine brain. The resulting fractions were analysed for Ca²⁺-binding and calmodulin activity. Three peaks of Ca²⁺-binding activity were observed, and only peak III co-eluted with calmodulin activity. The pooled peak I was applied to a hydroxyapatite column and eluted with a linear gradient of sodium phosphate. As shown in Fig. 2, only a single peak of Ca²⁺-binding activity was resolved between 50 mM and 80 mM-phosphate. The unbound fraction did not contain detectable Ca²⁺-binding activity. The Ca²⁺-binding activity peak was pooled, heat-treated (see the Experimental section), and subjected to gel-permeation chromatography (Fig. 3). A single peak of Ca²⁺-binding activity migrating with an apparent *M_r* of 35500 was resolved. By this purification procedure 33 mg of CAB-27 was purified from 1 kg of bovine brain. Determined by the densitometric analysis of SDS/PAGE gels, 1 kg of bovine brain contains 130 mg of CAB-27; therefore 25% of CAB-27 was recovered after purification (Table 1).

The SDS/PAGE analysis of the purification procedure is presented in Fig. 4. The purity of CAB-27 is suggested by the presence of a single protein band after the final step of purification. Protein purity was also confirmed by isoelectric focusing (Fig. 5a). ⁴⁵Ca²⁺ autoradiography confirmed that the purified protein bound Ca²⁺ (Fig. 5b).

Fig. 1. DEAE-cellulose chromatography of bovine brain 100000 g supernatant

Bovine brain 100000 g supernatant was chromatographed on DEAE-cellulose column as described in the Experimental section. Fractions (25 ml) were collected and analysed. ●, Ca^{2+} -binding activity; ×, NaCl concentration; □, calmodulin activity.

Fig. 2. Chromatography of peak I (Fig. 1) on hydroxyapatite

Hydroxyapatite chromatography of peak I from DEAE-cellulose chromatography was performed as described in the Experimental section. ●, Ca^{2+} -binding activity; ○, protein concentration; ×, phosphate concentration.

Physical properties of CAB-27

In a previous paper (Waisman *et al.*, 1983a) we erroneously reported the molecular mass of CAB-27 as 24000 Da. As shown in Fig. 4, calculation from SDS/PAGE analysis of CAB-27 established a value of 27000 Da.

Table 2 summarizes the various physical parameters of the purified CAB-27. A molecular mass of the protein, determined from the Stokes radius and sedimentation coefficient (Siegel & Monty, 1966), under non-denaturing conditions, of 35500 Da indicates that the protein is a monomer. The frictional ratio (Siegel & Monty, 1966) of 1.17 suggests that CAB-27 is a symmetrical molecule. By using a protein concentration determined by amino acid analysis, an absorption coefficient $A_{278}^{1\%} = 5.7$ was

calculated. The mean residue ellipticity in the absence of Ca^{2+} was $[\theta]_{222} = -6282$ (degrees $\cdot \text{cm}^2 \cdot \text{dmol}^{-1}$). Addition of Ca^{2+} did not affect the $[\theta]_{222}$ value. By using the computer-assisted program (Siegel *et al.*, 1980), the apparent α -helix content of CAB-27 was estimated to be about 16%.

Amino acid composition

The amino acid composition of CAB-27 is shown in Table 3. CAB-27 contains about 26% glutamic acid and aspartic acid and about 13% basic residues (lysine, histidine and arginine). The pI of 4.8 indicates that the molecule is highly acidic. Interestingly, this protein has a high ratio of phenylalanine/tyrosine and only 2 mol of tryptophan/mol of protein, which accounts for its

Fig. 3. High-performance gel-permeation liquid chromatography (TSK-G3000SW) of the Ca^{2+} -binding activity peak (Fig. 2) from hydroxyapatite chromatography of peak I

Heat treatment of Ca^{2+} -binding activity peak from hydroxyapatite chromatography of peak I, followed by high-performance gel-permeation liquid-chromatography were performed as described in the Experimental section. Fractions were analysed for Ca^{2+} -binding activity (●) and protein concentration (○).

Table 1. Purification of CAB-27 from bovine brain

Purification step	Total protein (mg)	Amount of CAB-27* (mg)	Yield (%)	Purification (fold)
1. 100000 g supernatant	41020	132	100	1.0
2. DEAE-cellulose	3025	82	62.3	8.5
3. Hydroxyapatite	176	53	40.3	71.6
4. Heat treatment	56	42	31.6	149.6
5. TSK-G3000SW	33	33	25.1	312.5

* As determined by densitometric analysis of SDS/PAGE.

characteristic u.v.-absorption spectrum (results not shown) and low $A_{278}^{1\%}$ value. The spectrum and unusual phenylalanine/tyrosine ratio is similar to the spectrum reported for calmodulin and other EF-hand-containing proteins (Table 3), such as calcineurin (β subunit), S-100 and vitamin-D-dependent Ca^{2+} -binding protein (28K-CaBP). Table 3 shows that, despite their similar molecular masses, 28K-CaBP and CAB-27 have similar but non-identical amino acid compositions. The differences include the presence of cysteine residues in CAB-27, but not in 28 K-CaBP, and a significantly higher content of valine and histidine in CAB-27.

Ca^{2+} -binding properties of CAB-27

Fig. 6 presents the saturation curve for Ca^{2+} binding to CAB-27. Scatchard (1949) analysis of data (Fig. 6, inset) reveals that, in the presence of 3.0 mM- MgCl_2 and 150 mM-KCl, CAB-27 binds 2.0 mol of Ca^{2+} /mol of

protein with an apparent $K_d = 0.22 \mu\text{M}$. In the absence of MgCl_2 the apparent K_d is decreased to $0.14 \mu\text{M}$ (results not shown). This result suggests that CAB-27 demonstrates both specificity and high affinity for Ca^{2+} *in vitro*, and therefore may represent an intracellular Ca^{2+} receptor *in vivo*.

Tissue distribution of CAB-27

The tissue distribution of CAB-27 was investigated by the immunoblot procedure (Towbin *et al.*, 1979). In Fig. 7, 200 μg of bovine brain and kidney 100000 g supernatants and 2 μg of purified CAB-27 were subjected to SDS/PAGE and then immunoblotted by using the mouse anti-CAB-27 antibody. The results suggest that the CAB-27 immunoreactivity present in the 100000 g supernatants of brain and kidney is of molecular mass identical with that of purified bovine brain CAB-27. Similar experiments using pre-immune antiserum failed

Fig. 4. SDS/PAGE analysis at different stages of CAB-27 purification

Portions of pooled fractions obtained during CAB-27 purification were subjected to SDS/PAGE (5–20% acrylamide gels). Lanes: *a*, bovine brain 100 000 *g* supernatant; *b*, DEAE-cellulose peak I; *c*, hydroxyapatite chromatography of peak I; *d*, after heat treatment; *e*, TSK-G3000SW chromatography of heat-treated peak I; *f*, *M_r* standards [alcohol dehydrogenase (150 000), catalase (116 000), phosphorylase *b* (97 400), bovine serum albumin (66 200), ovalbumin (45 000), carbonic anhydrase (29 000), trypsin inhibitor (20 100)].

to produce visible bands (results not shown). In addition, the 100 000 *g* supernatants of bovine heart, lung, spleen, skeletal muscle, smooth muscle, parathyroid and intestinal mucosa were also analysed by the immunoblot procedure, and only the 100 000 *g* supernatants of heart and spleen contained weak immunoreactivity (results not shown). These results suggest that CAB-27 may not have a broad tissue distribution. Work is now needed to quantify CAB-27 in the various bovine tissues by radioimmunoassay.

Inhibition of phospholipase A₂

Phospholipase A₂-inhibitory proteins have been detected in a number of systems, including rat macrophages (Blackwell *et al.*, 1982), rat renal medullary cells (Rothhut *et al.*, 1983), rabbit neutrophils (Hirata, 1981), and mouse and bovine thymus preparations (Gupta *et al.*, 1984). These proteins have been termed 'lipocortins' and shown to consist of a 35 kDa monomer (lipocortin I), a 36 kDa monomer (lipocortin II) or a 36 kDa oligomer consisting of two copies of both the 36 kDa and 6–10 kDa subunits (lipocortin-85). Lipocortin II has been shown to bind 2 mol of Ca²⁺/mol with *K_d*

Fig. 5. Agarose-gel isoelectric focusing of CAB-27 (*a*) and ⁴⁵Ca²⁺ autoradiographic analysis (*b*)

Agarose-gel isoelectric focusing was performed in the presence of 2 M-urea as described in the Experimental section. Separated peptides were electrophoretically transferred to the nitrocellulose sheet at the running current of 100 mA for 60 min. After ⁴⁵Ca²⁺ autoradiography, the nitrocellulose sheet was stained with Amido Black and destained for observation of peptide bands.

Table 2. Physical properties of CAB-27

Sedimentation coefficient (<i>s</i> _{20,w})	3.63 S
Stokes radius (nm)	2.30
Frictional coefficient (<i>f</i> / <i>f</i> ₀)	1.17
Partial specific volume (<i>V</i>)	0.733
Absorbance (<i>A</i> ₂₇₈ nm)	5.722
pI	4.8
Molecular mass	
From sedimentation and gel filtration	35 500 Da
From SDS/PAGE	27 000 Da
α-Helical content (%)	16.0

(Ca²⁺) of 4.5 μM (Glenney, 1986). Lipocortins have been shown to inhibit phospholipase A₂ activity, by both an inhibition assay *in vitro*, which utilizes purified pig pancreatic phospholipase A₂ (Pepinsky *et al.*, 1986), and in cellular assays in which prostaglandin production is monitored. Work from our laboratory using the phospholipase A₂ assay *in vitro* has identified lipocortin-85 as a potent inhibitor of phospholipase A₂ activity (Khanna *et al.*, 1986).

Western blot analysis has revealed that the lipocortins are not present in brain tissues (Saravis *et al.*, 1979). In contrast, 'Northern' analysis shows small but detectable amounts of lipocortin mRNA and protein in rat brain (Wallner *et al.*, 1986). We have used the phospholipase A₂ assay *in vitro* to test several bovine brain Ca²⁺-binding proteins as potential inhibitors of phospholipase A₂ activity. Under identical assay conditions used for the lipocortins (Khanna *et al.*, 1986), we have identified CAB-27 as an inhibitor of phospholipase A₂ activity. As

Table 3. Amino acid composition of CAB-27

	Composition (mol of residues/mol)					
	CAB-27 ^a	Calmodulin ^b	Parvalbumin ^c	28K-CaBP ^d	Calcineurin β^e	Calregulin ^f
Asx	24	23	12	33	28	90
Thr	9	12	5	12	3	20
Ser	13	4	5	13	11	20
Glx	40	27	13	38	22	97
Pro	10	2	1	8	3	35
Gly	17	11	9	17	14	37
Ala	20	11	11	15	5	25
Cys	3	0	0	0	0	3
Val	18	7	5	7	13	23
Met	4	9	3	5	6	5
Ile	8	9	6	11	11	24
Leu	26	9	9	33	14	24
Tyr	3	2	0	7	3	19
Phe	14	8	9	12	12	25
His	7	1	2	4	2	52
Lys	16	7	16	22	15	11
Arg	10	6	1	6	6	13
Trp	2	0	0	1	0	24
M_r	27000	16700	12000	28000	19000	63000

^a Values correspond to M_r of 27000.^b Watterson *et al.* (1980).^c Berchtold *et al.* (1982).^d Baudier *et al.* (1985).^e Aitken *et al.* (1984).^f Waisman *et al.* (1985b). **Fig. 6. Ca^{2+} binding by CAB-27**

The experiment was carried out by equilibrium dialysis as described in the Experimental section. Conditions: 150 mM-KCl, 10 mM-Mops (pH 7.1), 3.0 mM- MgCl_2 , 1.0 mM-DTT and 0.1 mM-EGTA. Inset, Scatchard plot of data: $\bar{V}$, Ca^{2+} bound (mol/mol of CAB-27); C , free $[\text{Ca}^{2+}]$.

Fig. 7. Distribution of CAB-27 immunoreactivity in bovine tissues

Bovine tissue 100000 *g* supernatants (200 μg) or purified bovine brain CAB-27 (2 μg) were electrophoresed on 12.5%-polyacrylamide gel in the presence of 0.1% SDS. Mouse antibodies to CAB-27 or control antibodies (results not shown) were used in the immunoblotting procedure as outlined in the Experimental section. The lanes correspond to purified bovine brain CAB-27 (a), bovine brain 100000 *g* supernatant (b) and bovine kidney 100000 *g* supernatant (c).

Fig. 8. Inhibition of phospholipase A_2 by bovine brain Ca^{2+} -binding proteins

Various Ca^{2+} -binding proteins were tested for phospholipase A_2 -inhibitory activity as described in the Experimental section. The data presented show a typical titration curve for CAB-27 (●), or a single analysis in triplicate with 60 μg of CAB-48 (×), calmodulin (○), calregulin (□), S-100 (▽), calcineurin (◊), or parvalbumin (△).

shown in Fig. 8, CAB-27 inhibits phospholipase A_2 activity in a dose-dependent manner. The amount of CAB-27 required for half-maximal inhibition of 7.2 pmol of pig pancreatic phospholipase A_2 was about 800 pmol. In contrast, about 44 pmol of lipocortin II (N. C. Khanna, unpublished work) was required for half-maximal inhibition. The inhibition of phospholipase A_2 activity by CAB-27 was reversible, since immunoprecipitation of CAB-27 by a polyclonal antibody completely restored phospholipase A_2 activity. Polyclonal antibodies to lipocortin I or lipocortin II (generously given by Dr. Blake Pepinsky, Biogen Research Corp., Cambridge, MA, U.S.A.) did not cross-react with CAB-27. Consistent with the observations of others, we have found that, with the inhibitor assay *in vitro*, the maximal inhibition of phospholipase A_2 activity by lipocortins (Khanna *et al.*, 1986; Pepinsky *et al.*, 1986) or CAB-27 (Fig. 8) is about 60%. The reason for the incomplete inhibition of phospholipase A_2 activity by lipocortins or CAB-27 is at present unclear.

The inhibition of phospholipase A_2 activity by bovine brain Ca^{2+} -binding proteins was specific for CAB-27, since other major Ca^{2+} -binding proteins, such as calmodulin, calregulin, S-100, parvalbumin, CAB-48 and calcineurin, did not inhibit phospholipase A_2 activity (Fig. 8). Considering the relatively poor inhibitory action of CAB-27, in comparison with lipocortin, and the paucity of information available on the properties of bovine brain phospholipase A_2 , it is at present not possible to determine if inhibition of phospholipase A_2 by CAB-27 represents a phenomenon *in vivo*. Further studies will therefore be required to identify the biological activity of CAB-27.

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