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Structural and functional characterization of endonexin II, a calcium- and phospholipid-binding protein

(annexins/lipocortin/calpactin)

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ABSTRACT **A protein with an apparent M_r of 33,000 was previously purified from the EGTA eluate of a human placental particulate fraction. We now report the amino acid sequence of approximately one-third of this protein and show that it has extensive homology with a newly defined family of Ca^{2+}-binding proteins termed annexins. The partial sequence of the placental protein could be aligned with the sequence of either lipocortin I or calpactin I such that 49% and 58%, respectively, of the residues were identical. A comparison of the partial sequences of the placental protein with the partial sequence of bovine endonexin revealed 74% sequence identity. Based on this close relationship, the placental protein was named endonexin II. Equilibrium dialysis showed that endonexin II bound Ca^{2+} (K_d >0.5 mM) and the affinity was increased by phosphatidylserine liposomes ($K_d \simeq 100$ μM). In addition, endonexin II bound to phosphatidylserine- and phosphatidylethanolamine-containing liposomes in a Ca^{2+}-dependent manner, and the binding was cooperative with respect to Ca^{2+} concentration (Hill constant >3). The Ca^{2+}- and phospholipid-binding properties of endonexin II raise the possibility that each of the four internally repeated sequences that have been demonstrated within this family of proteins contains a Ca^{2+}-binding site.**

Recent research has revealed the existence of a family of proteins termed annexins with an apparent M_r of ≈35,000 that undergo Ca^{2+}-dependent binding to phospholipids (1). Members of this family include proteins initially investigated as substrates for protein-tyrosine kinases, mediators of exocytosis, or components of the cytoskeleton, but their exact physiological roles are not yet known. The realization that they were related was the unexpected result of structural studies. The complete amino acid sequences of two of these proteins, lipocortin I (2) and calpactin I (3–5), have been deduced from cDNA clones: lipocortin I and calpactin I also have been called calpactin II (6) and lipocortin II (5), respectively. These proteins have ≈50% overall sequence homology but have only limited homology (14%) in the N-terminal domain containing 54 amino acids. The N-terminal domains are not required for Ca^{2+} or phospholipid binding (7, 8). The larger core domains contain a 4-fold internal repeat containing a highly conserved 17-amino acid sequence termed the consensus sequence (3, 5). In addition, a bovine protein called endonexin has been partially sequenced and shown to contain four of these consensus regions (9). Partial sequence information of *Torpedo* calelectrin and protein II shows that these proteins also contain this consensus sequence (9). Other proteins, including calcimedins (10), chromobindins (11), and a variety of proteins with an apparent M_r of ≈70,000 (10, 11), may be related structurally; however, sequence information is not yet available. None of these proteins appears to be related structurally to the Ca^{2+}-binding protein calmodulin or the Ca^{2+}/phospholipid-dependent enzyme protein kinase C.

Lipocortin I (12–16) and calpactin I (17, 18) are substrates for the protein-tyrosine kinase activities associated with the epidermal growth factor receptor and $pp60^{src}$, respectively, which raises the possibility that these proteins are involved in the regulation of cellular proliferation. However, neither the physiological roles of these proteins nor the effect of phosphorylation on their activities is known. Lipocortin I inhibits phospholipase A_2 in an *in vitro* assay and has been proposed to mediate the steroid-induced antiinflammatory response (2, 19). However, two independent studies have shown that the phospholipase A_2 inhibition in this assay is secondary to Ca^{2+}-dependent binding to the lipid substrate and not by way of direct interaction with the enzyme (16, 20). Thus, lipocortin I has not yet been shown to be a physiological inhibitor of phospholipase A_2.

While purifying lipocortin I from EGTA extracts of a placental particulate fraction, we also purified a M_r 33,000 protein, "peak G," that inhibited phospholipase A_2 in an *in vitro* assay (16). Peak G was not immunologically related to lipocortin I or calpactin I and was not a substrate for the epidermal growth factor-stimulated protein-tyrosine kinase (16). We now report that peak G has extensive sequence homology with the family of Ca^{2+}-binding proteins that contain the 17-amino acid consensus region. It shows greatest homology with endonexin (9), so it has been named endonexin II. The Ca^{2+}- and phospholipid-binding properties of endonexin II were investigated.

MATERIALS AND METHODS

Materials. Endonexin II (peak G) and lipocortin I ("peak B") were purified from human placenta as described (16). The concentration of endonexin II was determined by the method of Lowry *et al.* (21) using bovine serum albumin as a standard. Endonexin II was iodinated to a specific activity of 24,100 cpm/ng exactly as described for lipocortin I (16). The ^{125}I-labeled endonexin II was analyzed by $NaDodSO_4$/PAGE (22) and autoradiography and showed a single radioactive band with an apparent M_r of 33,000. The following phospholipids were purchased from Sigma; phosphatidylserine (PtdSer) (bovine brain, P8518), phosphatidylcholine (PtdCho) (egg yolk, P6386), and phosphatidylinositol (PtdIns) (soybean, P5766).

Protein Sequence Analysis. Prior to analysis, purified endonexin II was dissolved in 0.1% trifluoroacetic acid, applied to a Vydac C_4 column (0.41 × 25 cm), and eluted with a linear gradient (5–80%) of acetonitrile over 60 min. Proteins were

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Abbreviations: PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine.

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digested with CNBr (500-fold molar excess) in the dark for 22 hr at room temperature in 70% formic acid. Certain samples were reduced and alkylated before CNBr digestion by dissolving ≈2 nmol of endonexin II in 1 ml of Tris buffer (50 mM, pH 8.3) containing guanidine hydrochloride (6 M) and incubating at 37°C in the presence of 10 mM dithiothreitol for 1 hr. This was followed by incubation in 22 mM iodoacetamide for 1 hr at 37°C in the dark. The protein was isolated by reverse-phase chromatography as described above. Samples for amino acid sequence analysis were applied directly from the HPLC fraction to Polybrene-treated filters. The samples were subjected to automated Edman degradations using an Applied Biosystems (Foster City, CA) 470A instrument and a standard program supplied by the manufacturer. Phenylthiohydantoin amino acids were identified by HPLC using an Applied Biosystems model 120 analyzer.

Ca^{2+}-Induced Difference Spectrum. The spectrum of endonexin II [1.4 mg of protein per ml in 20 mM Hepes buffer (pH 7.4) containing 25 μM EGTA] was recorded using a Cary 17D spectrophotometer interfaced with an OLIS computer system. $CaCl_2$ then was added so that the ionized free Ca^{2+} concentration was calculated to be 30 μM if the binding of Ca^{2+} to protein was ignored. The difference spectrum was calculated by subtracting the initial spectrum from the spectrum recorded at 30 μM free Ca^{2+}.

Equilibrium Dialysis. Endonexin II (135 μg/40 μl) in the presence or absence of PtdSer (30 μg) was dialyzed against 0.5 liter of buffer B (20 mM Hepes/100 mM KCl/2 mM $MgCl_2$/2 mM NaN_3, pH 7.4) containing $^{45}Ca^{2+}$ (50 μCi/liter; 1 Ci = 37 GBq) and the indicated concentration of ionized Ca^{2+} was standardized by a Ca^{2+}-sensitive electrode as described (8).

Phospholipid Vesicles. PtdSer liposomes used in the equilibrium dialysis experiments were prepared by sonication in buffer B (8). Large thin-walled vesicles used to measure ^{125}I-labeled endonexin II (^{125}I-endonexin II) binding to phospholipid were formed in the presence of 240 mM sucrose by the method of Reeves and Dowben (23) using either PtdCho alone or equal molar mixtures of PtdCho and the indicated phospholipid. The vesicles were suspended in buffer P (20 mM Hepes/100 mM NaCl/2 mM $MgCl_2$/2 mM NaN_3, pH 7.4) and pelleted by centrifugation (15 min, 20,000 × *g*).

Binding Experiments. The binding of ^{125}I-endonexin II (0.5 μg, 20,000 cpm) to phospholipid vesicles (30 μg) was performed in buffer P (0.1 ml) as described (8). The binding of ^{125}I-endonexin II (0.25 μg, 15,000 cpm) to chicken F-actin (50 μg) was measured in buffer B (0.1 ml) containing bovine serum albumin (0.1 mg) as described (8).

RESULTS

Structural Studies of Endonexin II. We previously purified a protein of apparent M_r 33,000, peak G, from the EGTA eluate of a placental particulate fraction that was prepared in the presence of Ca^{2+} (16). Electrophoretic transfer blot analysis of placental tissue extracted under denaturing conditions showed a major band that was immunoreactive with antiserum against peak G. This band had the same apparent molecular weight as the isolated protein, indicating that peak G did not undergo any detectable proteolysis during isolation (data not shown). Because amino acid sequence analysis (see below) indicates that this protein is closely related to endonexin (9), we propose that peak G be called endonexin II.

The amino acid composition of acid-hydrolyzed human endonexin II was determined (data not shown). Its composition was very similar to the compositions of *Torpedo* calelectrin (24), protein II (25), and the protein with an apparent M_r of 32,500 (24) that was recently named endonexin (9).

When endonexin II was subjected to automated Edman degradation, no sequential release of phenylthiohydantoin amino acids was observed even when as much as 1.0 nmol of protein was placed in the sequenator. Either native protein or reduced and alkylated protein was digested with CNBr and the cleavage products were separated. Amino acid sequence data were obtained for four of the major peptides from these digests (Table 1) and amounts to 104 residues or approximately one-third of the amino acids of this M_r 33,000 protein. The partial sequence shows striking homology with the sequences of a family of proteins that associated with phospholipids in a Ca^{2+}-dependent manner (see *Discussion*).

Ca^{2+}-Binding Properties of Endonexin II. Because endonexin II is related structurally to other proteins that bind Ca^{2+} (see *Discussion*), and because it associated with the particulate fraction of placenta in a Ca^{2+}-dependent manner, its Ca^{2+}-binding properties were investigated. To determine whether purified endonexin II underwent a conformational change as a consequence of Ca^{2+} binding, a Ca^{2+}-induced difference spectrum was recorded. Ca^{2+} caused a decrease in absorbance in the 275- to 295-nm region of the spectrum with a minimum at 287 nm (Fig. 1). This is indicative of aromatic amino acid side chain exposure to a more polar environment (31).

The characteristics of Ca^{2+} binding to endonexin II also were investigated by equilibrium dialysis against $^{45}Ca^{2+}$. The dialysis buffer contained 2 mM Mg^{2+} to reduce the possibility of detecting nonspecific divalent cation binding. Endonexin II had a low affinity for Ca^{2+} but the affinity was increased significantly in the presence of PtdSer liposomes (Fig. 2). Half-maximal binding occurred at 101 μM Ca^{2+} and ≈5 mol of Ca^{2+} bound per mol of endonexin II (Fig. 2). The Hill constant (27) for $^{45}Ca^{2+}$ binding was 1.9, indicating that two or more binding sites interact in a cooperative manner (Fig. 2).

Phospholipid-Binding Properties of Endonexin II. The Ca^{2+}-dependent binding of endonexin II to phospholipids was investigated by measuring the ability of ^{125}I-endonexin II to cosediment with phospholipid vesicles when subjected to centrifugation. In the absence of phospholipid, ^{125}I-endonexin II was not found in the pellet even in the presence of high Ca^{2+}. In the absence of Ca^{2+}, ^{125}I-endonexin II did not copellet with phosphatidylethanolamine (PtdEtn) or PtdSer vesicles; however, >90% of the added radioactivity was in the pellet when the concentration of Ca^{2+} was >80 or >120

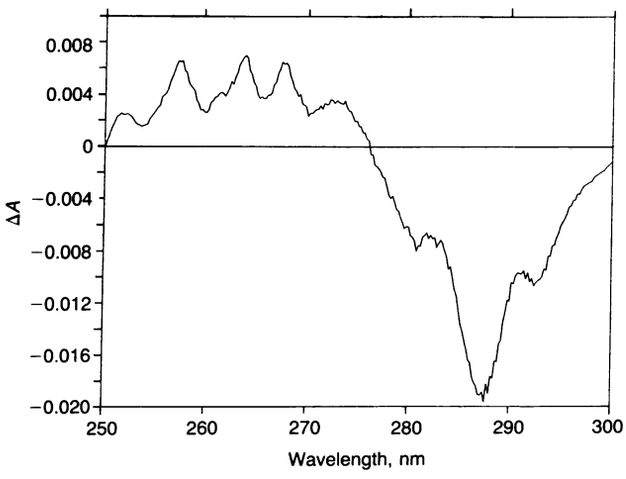

FIG. 1. Ca^{2+}-induced difference spectrum of endonexin II. The spectrum of endonexin II was recorded in the presence of EGTA. $CaCl_2$ then was added so that the ionized free Ca^{2+} concentration was 30 μM and the difference spectrum was calculated by subtracting the initial spectrum from the spectrum in the presence of Ca^{2+}.

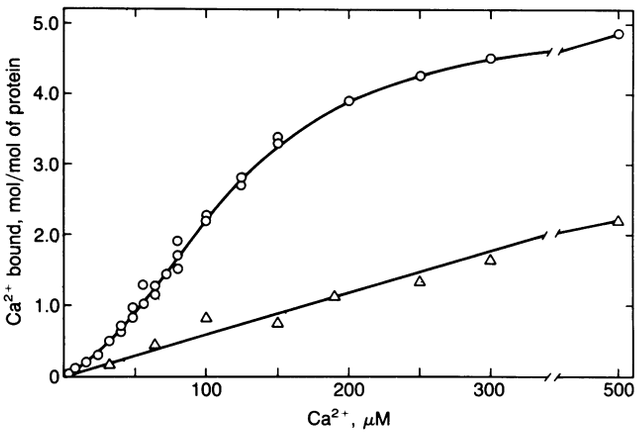

FIG. 2. $^{45}Ca^{2+}$ binding by endonexin II in the presence and absence of PtdSer. Endonexin II was subjected to equilibrium dialysis against the indicated concentration of free Ca^{2+} in buffer B in the presence (○) or absence (△) of PtdSer liposomes and the binding of $^{45}Ca^{2+}$ was determined. Each point is the average of quadruplicate determinations after subtracting the background as determined in control reactions in the absence of protein. The background was 30–50% of total binding, with the highest value occurring at the highest Ca^{2+} concentration. The data are consistent with the results of five separate experiments. The data for $^{45}Ca^{2+}$ binding in the presence of PtdSer between 16 and 300 μM Ca^{2+} was replotted by the method of Hill (27). The data were fit to a straight line by least squares analysis ($R^2 = 0.99$) and had a slope of 1.9 and an x intercept of 101 μM.

μM, respectively (Fig. 3). Half-maximal binding to PtdEtn or PtdSer vesicles occurred at 40 and 53 μM Ca^{2+}, respectively, and both binding curves are strikingly sigmoidal. The Hill constants for binding to PtdEtn and PtdSer vesicles were 3.4 and 3.1 (Fig. 3), respectively, indicating more than three binding sites for Ca^{2+} that interact in a cooperative manner.

The Ca^{2+}-dependent binding of endonexin II to phospholipid vesicles showed specificity with regard to the polar head group of the lipid. Less than 1% of the added ^{125}I-endonexin II associated with PtdCho vesicles even in the presence of high Ca^{2+} (0.5 mM). Endonexin II bound nearly quantitatively to vesicles made from a mixture of PtdCho and PtdIns (1:1) but high concentrations of Ca^{2+} were required; half-maximal binding occurred at ≈300 μM Ca^{2+}.

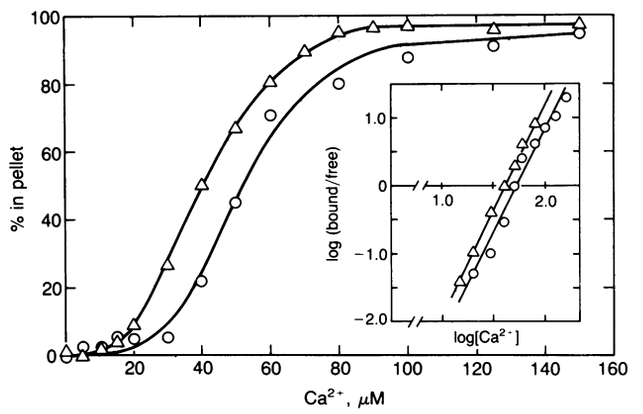

FIG. 3. Binding of ^{125}I-endonexin II to liposomes as a function of Ca^{2+} concentration. ^{125}I-endonexin was incubated in buffer P containing either PtdSer (○) or PtdEtn (△) and the indicated concentration of Ca^{2+}. The percentage of added radioactivity that was present in the washed lipid pellet was determined and each point is the average of duplicate determinations. Less than 10% of the added radioactivity was in the pellet in control reactions containing up to 1 mM Ca^{2+} but no phospholipid. The binding data for PtdSer (20–150 μM Ca^{2+}) and PtdEtn (15–80 μM Ca^{2+}) were replotted by the method of Hill (27) in the *Inset*. The data were fit to a straight line by least squares analysis. The curve for binding to PtdSer had a slope of 3.1 and an x intercept of 53 μM and the curve for binding to PtdEtn had a slope of 3.4 and an x intercept of 40 μM.

To determine whether iodination of endonexin II altered the phospholipid-binding properties of the protein, the experiments in this section were performed using native endonexin II. The Ca^{2+}-dependent association of the protein with the pellets of the different phospholipids was evaluated by $NaDodSO_4$/PAGE (22) followed by staining with Coomassie blue. The results obtained using this method (not shown) were indistinguishable from the results obtained using ^{125}I-endonexin II (Fig. 3).

Association of ^{125}I-Endonexin II with F-Actin. Since other proteins that share sequence homology with endonexin II associate with filamentous actin in a Ca^{2+}-dependent manner (6, 8, 25), endonexin II was tested for this property using described methods (8). ^{125}I-Endonexin did not cosediment with F-actin when subjected to centrifugation in the absence of Ca^{2+}, but increasing amounts of radioactivity were detected in the pellet with increasing Ca^{2+} concentrations. At 0.10, 0.25, 0.50, 1.0, and 2.0 mM Ca^{2+}, 12%, 16%, 30%, and 40% of the added ^{125}I-endonexin II were in the pellet. The binding properties were the same when performed in the presence of PtdSer-containing liposomes. The high concentration of Ca^{2+} required for association with actin raises doubts about its physiological significance. However, it should be noted that the putative actin-binding region of the Ca^{2+}-dependent actin-binding protein gelsolin (28) shares homology with endonexin II.

DISCUSSION

We previously identified a M_r 33,000 protein, peak G, that underwent reversible Ca^{2+}-dependent binding to the high-speed pellet from human placenta (16). This protein inhibited phospholipase A_2 in an *in vitro* assay (16). Because of structural similarities to bovine endonexin (9), peak G has been named endonexin II (see below). To avoid confusion, bovine endonexin will be called "endonexin I."

Structural Relationship of Endonexin II to Other Proteins. The amino acid sequence of 104 residues of endonexin II was determined (Table 1) and was compared to the amino acid sequences of proteins that comprise a family of Ca^{2+}-binding proteins (1). The physiological role of these proteins is not known but they share the common property of binding to phospholipid in a Ca^{2+}-dependent manner. They have extensive sequence homology, particularly within a 17-amino acid consensus sequence that repeats four times within each protein (1, 9). The partial sequence of endonexin II could be aligned with the sequence of human lipocortin I (2) and

Table 1. Sequence data for endonexin II

Peptide	Sequence
1	KGAGTDDHTLIRV
2	IKGDTSGDYKKALLLLXGEDD
3	VSRSEIDLFNIRKEFRKNFATSLY
4	KPSRLYDAYELKHALKGAGTNEKVLTEIIASRTPEELRAIKQVYEEW

The amino acid sequences of endonexin II peptides were determined in a gas-phase sequenator. Peptide 1 was generated by treatment of endonexin II with CNBr and peptides 2–4 were generated by treatment of reduced and alkylated endonexin II with CNBr. Peptide 2 also was obtained and sequenced from the reduced and alkylated digest and peptide 1 also was obtained and sequenced from the native digest. None of the peptides appeared to be sequenced to completion. The sequential release of phenylthiohydantoin amino acids from these peptides occurred with initial yields of 55–93%; repetitive yields were 90–93%.

calpactin I (5), two proteins whose complete sequences are known, such that 51 and 60 amino acids, respectively, were identical with the 104 amino acids of endonexin II (Fig. 4). Of the 104 amino acid residues, 46 positions contained the same residue in endonexin II, lipocortin I, and calpactin I, implying that these three proteins share a conserved function (Fig. 4). The partial sequence of endonexin II included 2 of the 17 amino acid consensus sequences and the homology was higher within this region. It is reasonable to speculate that all of the M_r 30,000–40,000 proteins in this family contain 4 consensus sequences and that 2 more consensus sequences will be found in endonexin II when the entire structure is elucidated.

Partial sequence information consisting mostly of the 17-amino acid consensus sequence region is available for three other related proteins and these sequences are compared with the two consensus regions of endonexin II in Table 2. A partial sequence of bovine endonexin I including four 17-amino acid repeat sequences has been determined (9). One of the consensus regions of endonexin I could be aligned with one of the consensus regions of endonexin II such that 15 of the 17 amino acid residues were identical. Two other peptides could be aligned such that there was 71% identity. By comparison, there was 62% and 65% identity within this 17-amino acid consensus region of endonexin II with lipocortin I and calpactin I, respectively. Lipocortin I and calpactin I share 79% identity within this region. Like lipocortin I and calpactin I, endonexins I and II may be less homologous outside of the consensus region.

Endonexin II also shares homology with the 17-amino acid consensus regions within *Torpedo* calelectrin and protein II (Table 2). Since all four of these regions are not available for these proteins, it is difficult to speculate on the extent to which they are related to endonexin II.

Structural, functional, and immunological data indicate endonexin II is not simply a human homologue of bovine endonexin I. Comparison of the partial sequences of endonexins I and II indicates that they share ≈74% sequence identity, which is lower than one would expect for species variation: bovine, murine, and human calpactin I share ≈98% identity (3–5). Also, bovine endonexin I had a high affinity for PtdIns but did not associate with PtdSer (9), whereas endonexin II had a high affinity for PtdSer and only associated with PtdIns at very high Ca^{2+} concentrations. Finally, endonexin II showed weak cross-reactivity on electrophoretic transfer blots with antiserum against bovine endonexin I, whereas the uncharacterized "peak D" protein from the DEAE fraction of the placental extract (16) showed strong reactivity (D.D.S. and H.T.H., unpublished results).

Several Ca^{2+}-binding proteins known as chromobindins have been identified in chromaffin granules (11). The apparent molecular weight and charge of endonexin II and chromobindin 7 are very similar.

Table 2. Comparison of consensus sequences of endonexin II and other proteins

Comparison protein	Endonexin II consensus sequence peptides	
	KGAGTNEKVLTEIIASR	KGAGTDDHTLIRVMVSR
Endonexin I	X–L––D–DAIINVL–Y–	X–L–––EDAI–N–LAY–
Endonexin I	––L––DDDT–IRVMV––	––L––––D–––––––––
Endonexin I	–––––D–ST–I––L–––	–––––––ES–––EILA––
Endonexin I	X––––D–GS–I––L–––	X––––––EGS––EILA––
Calelectrin	X––––S–N––I––L–––	X––––SENV––EILA––
Calelectrin	X–F––D–D–ILDLLTQ–	X–F–––EDVILDLLTQ–
Protein II	––L––D–GAIIDVL–YX	––L–––EGAI–D–LAYX

The two 17-amino acid consensus sequence regions of endonexin II (Fig. 4) are compared with consensus sequences from endonexin I, *Torpedo* calelectrin, and protein II that were determined by Geisow *et al.* (9). Positions at which the comparison protein contains a residue identical to that of endonexin II are marked with "–" and unknown residues are marked with "X." The methionine residue in one of the endonexin II peptides was assigned based on the known specificity of CNBr cleavage.

Ca^{2+}-Binding Properties of Endonexin II. The Ca^{2+}-binding properties of endonexin II showed a number of parallels with calpactin I and lipocortin I as well as some differences. Like these other proteins (7, 8), the affinity of Ca^{2+} binding to endonexin II was enhanced in the presence of PtdSer (Fig. 2). However, the affinity was still lower by a factor of about 5–10 for endonexin II (half-maximal binding at 101 μM Ca^{2+}) than for the other two proteins. Also, Ca^{2+} bound to endonexin II in a cooperative manner, whereas the Ca^{2+}-binding curves for calpactin I (7) and lipocortin I (8) did not reveal any cooperativity.

Association of Endonexin II with Phospholipid. Like the other proteins in this family of Ca^{2+}-binding proteins, endonexin II binds to phospholipid in a Ca^{2+}-dependent manner. It shows its own unique specificity with regard to the polar head group of the phospholipid required for binding. Comparison of the different Ca^{2+}/phospholipid-binding properties of the proteins in this family with their structural differences should prove useful in determining how the binding sites are formed. The Ca^{2+}-binding site appears to be formed in a unique manner; these proteins show no sequence homology with other Ca^{2+}-binding proteins such as calmodulin and troponin C that contain "EF hands" (26). It will be particularly interesting to determine whether the 17-amino acid consensus region that repeats four times within these proteins is actually the Ca^{2+}-binding site. Equilibrium dialysis against $^{45}Ca^{2+}$ indicated that endonexin II binds 5 mol of Ca^{2+} per mol of protein (Fig. 2). Since this value is within experimental error of 4, we propose that one Ca^{2+} is bound

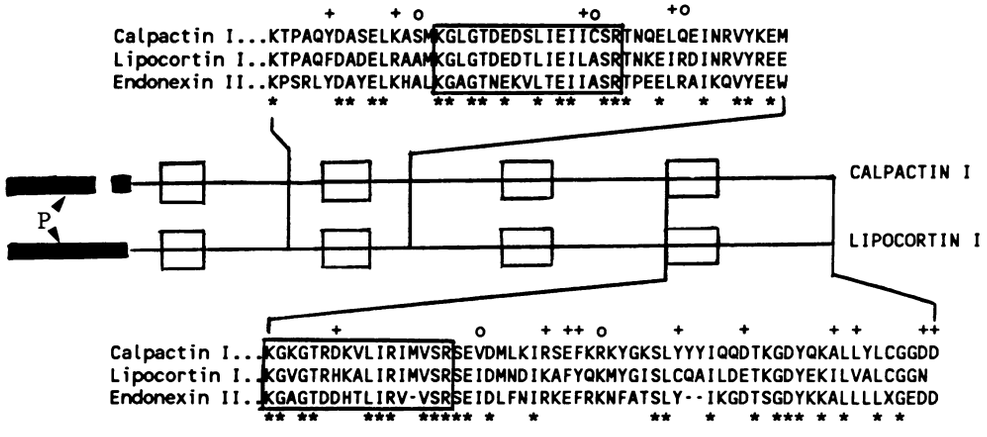

FIG. 4. Comparison of amino acid sequences of endonexin II, lipocortin I, and calpactin I. The N-terminal regions (thicker lines) of lipocortin I (2) and calpactin I (3–5) share only limited homology and contain the phosphorylation sites (P). The boxes mark the locations of the 17-amino acid consensus sequences. Positions containing residues with triple identity (*) and with only double identity between endonexin I and either lipocortin I (○) or calpactin I (+) are indicated.

to each of the four repeating domains. Previous studies on lipocortin I and calpactin I presented data in favor of a binding stoichiometry of both 2 (7, 14) and 4 (8) mol of Ca^{2+} per mol of protein. The fact that the Hill constant for the Ca^{2+}-dependent binding of endonexin II to phospholipid was >3 (Fig. 2) is a strong indication that three or more Ca^{2+}-binding sites interact in a cooperative manner in the formation of the ternary complex of endonexin II:Ca^{2+}:phospholipid and supports the hypothesis that a binding site can be formed within each of the four repeated regions in this family of proteins.

Comparison of the equilibrium binding (Fig. 2) and phospholipid association (Fig. 3) curves of endonexin II reveals that maximal binding to phospholipid occurred when only half of the Ca^{2+}-binding sites were occupied. This suggests that only two of the four Ca^{2+}-binding sites must be occupied to assume the conformation that binds phospholipid with high affinity. The fact that the effect of Ca^{2+} on phospholipid binding saturates at a concentration lower than that required for saturation of the Ca^{2+}-binding sites may explain why the Hill constants for these two processes are somewhat different (Figs. 2 and 3). Endonexin II appears to be capable of sharply altering its functional properties in response to a small change in Ca^{2+} concentration.

Annexins. Geisow proposed (29) the name "annexins" for the family of Ca^{2+}- and phospholipid-binding proteins that includes the following proteins with known sequence homology: endonexin I, lipocortin I, calpactin I, *Torpedo* calelectrin, and protein II. In this report we add placental endonexin II to this growing list. Lipocortin I (5, 14, 15, 30) and calpactin I (5) have been shown to be abundant placental proteins and immunological data suggest that endonexin I is also present (D.D.S. and H.T.H., unpublished results). Taken together these annexins constitute ≈1% of the total placental protein. The fact that certain proteins in this family are substrates for protein-tyrosine kinases raises the possibility that these interactions are modulated in a growth-dependent manner. Though endonexin II is not a substrate for the epidermal growth factor-stimulated kinase in A431 membranes (16), additional experiments are required to determine whether it is phosphorylated by other kinases.

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