Biochem. J. (1990) 266, 195–200 (Printed in Great Britain)

Characterization of Ca^{2+}-dependent phospholipid binding, vesicle aggregation and membrane fusion by annexins

R. Alexander BLACKWOOD*‡§ and Joel D. ERNST†‡

Departments of *Pediatrics and †Medicine and ‡Rosalind Russell Arthritis Research Laboratory, San Francisco General Hospital, University of California, San Francisco, CA 94143-0868, U.S.A.

The annexins are a family of structurally similar, Ca^{2+}-dependent, phospholipid-binding proteins. We compared six members of this family (calpactin I heavy chain, lipocortins I and III, endonexin II, p68 and protein II) to determine their phospholipid-binding specificities, as well as their ability to promote aggregation and fusion of phospholipid vesicles. The Ca^{2+} requirement for all of the proteins was lowest for binding to vesicles composed of phosphatidic acid, followed by phosphatidylserine and then phosphatidylinositol. Only protein II, p68, lipocortin III and endonexin II bound to vesicles composed of phosphatidylethanolamine, and none bound to phosphatidylcholine. Both calpactin I heavy chain and lipocortin I promoted aggregation of phosphatidylserine- or phosphatidylinositol-containing vesicles in the presence of less than 10 μM-Ca^{2+}. Lipocortin I promoted fusion of liposome membranes by lowering threshold Ca^{2+} concentrations. Although calpactin I heavy chain did not affect threshold Ca^{2+} concentrations, it did increase the rate and extent of spontaneous fusion. In contrast, p68 inhibited fusion at threshold Ca^{2+} concentrations. Whereas previous reports have emphasized properties that the annexins have in common, these findings reveal quantitative and qualitative differences among the annexins which may relate to distinct intracellular functions.

INTRODUCTION

Annexins are proteins that share the ability to associate with membrane phospholipids in a Ca^{2+}-dependent manner. In addition to this common functional property, the annexins are also structurally related: each contains at least four repeats of a conserved 70-amino-acid sequence (Brugge, 1986). Members of this family that have been identified in mammalian cells or tissues include calpactin I, lipocortin I, protein II, lipocortin III, p68, endonexin II and synexin (Glenney, 1986; Huang *et al.*, 1986; Saris *et al.*, 1986; Wallner *et al.*, 1986; Weber *et al.*, 1987; Crompton *et al.*, 1988*a*; Pepinsky *et al.*, 1988; Südhof *et al.*, 1988; Burns *et al.*, 1989).

Although no definite function has been established for any member of the annexin family, the observation that annexins interact with membranes in a Ca^{2+}-dependent manner has prompted the suggestion that they may participate in Ca^{2+}-regulated membrane traffic, such as in exocytosis (Burgoyne, 1988; Crompton *et al.*, 1988*b*). During exocytosis, secretory vesicle membranes bind to, and fuse with, the plasma membrane. In cells specialized for regulated secretion, this is often preceded by increases in cytosolic ionized Ca^{2+} concentrations. Because of their Ca^{2+}-dependent, membrane-binding properties, the annexins have been implicated as putative mediators of such interactions. Both calpactin I complex (Drust & Creutz, 1988) and synexin (Creutz *et al.*, 1979) promote the fusion of adrenal chromaffin granules at micromolar Ca^{2+} concentrations in the presence of Mg^{2+} and arachidonic acid. Synexin promotes the Ca^{2+}-dependent fusion of phosphatidylserine (PS) or phosphatidic acid (PA)/phosphatidylethanolamine (PE) vesicles at Ca^{2+} concentrations of 1 mM and 10 μM respectively in the presence of 1.5 mM-Mg^{2+} (Hong *et al.*, 1982). It also potentiates the fusion of polymorphonuclear leucocyte specific granules with PS vesicles in a Ca^{2+}-dependent manner in the presence of Mg^{2+} and arachidonic acid (Meers *et al.*, 1987).

Although annexins have all been found to have certain structural and functional features in common, we wished to determine whether individual annexins possess distinct properties. To characterize further the interactions between individual annexins, Ca^{2+} and phospholipids, we compared six monomeric annexins with respect to their Ca^{2+} requirements and phospholipid-binding specificities. We also examined their ability to promote aggregation of multilamellar liposomes and fusion of large unilamellar vesicles. We found that individual annexins differ with respect to the concentrations of Ca^{2+} they require for binding to phospholipids. In addition, we determined that the Ca^{2+} concentration required for binding to phospholipids is highly dependent on the head group of the phospholipid. We also found that whereas calpactin I heavy chain and lipocortin I possess the 'synexin-like' property of promoting aggregation of

Abbreviations used: PS, phosphatidylserine; PA, phosphatidic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-PE; Rh-PE, *N*-(lissamine rhodamine B sulphonyl)-PE; LUV, large unilamellar vesicles. Multiple names have been given to several of the annexins. The names used in this paper are those given in the first report of the full-length amino acid sequence for the given protein. Alternative names are presented in tables that can be found in Geisow *et al.* (1987), Klee (1988) and Haigler *et al.* (1989).

§ To whom correspondence should be addressed, at: University of California, Box 0868, San Francisco, CA 94143-0868, U.S.A.

phospholipid vesicles, endonexin II, lipocortin III, p68 and protein II do not exhibit this activity under the conditions of our assay. Finally, we found that although both lipocortin I and calpactin I heavy chain promote fusion of phospholipid vesicles, they do so by different mechanisms.

MATERIALS AND METHODS

Chemicals

PA (egg lecithin), phosphatidylcholine (PC, bovine brain), PS, phosphatidylinositol (PI) and PE (all from bovine liver), as well as *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE) and *N*-(lissamine rhodamine B sulphonyl)phosphatidylethanolamine (Rh-PE) were obtained from Avanti Polar Lipids, Birmingham, AL, U.S.A. H.p.l.c.-grade water was purchased from Fisher Scientific, Fairlawn, NJ, U.S.A. $CaCl_2$ hydrate (> 99.99%) was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. EGTA (puriss grade, > 99%) was obtained from Fluka Chemical Corp., Ronkonkoma, NY, U.S.A. All other chemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Fresh human placentas were obtained from the Obstetrics Department, San Francisco General Hospital. Snap-frozen human liver and spleen were obtained from The National Disease Research Interchange, Philadelphia, PA, U.S.A.

Ca^{2+} buffers

A series of Ca^{2+} stock solutions were prepared in buffer A (50 mM-Hepes/100 mM-KCl/1 mM-EGTA, pH 7.0) with a range of final ionized Ca^{2+} concentrations as predicted using the system of Bers (1982). The ionized Ca^{2+} concentration of a 1:4 dilution of each stock solution was measured with a Ca^{2+}-selective electrode (Orion Research, Inc., Boston, MA, U.S.A.). Ionized Ca^{2+} concentrations of the 1:4 dilutions ranged between 0.44 and 450 μM. The buffer concentration was chosen such that the measured pH of Ca^{2+} stock solutions changed by less than 0.03 pH units after 1:4 dilution with buffer A throughout the range of Ca^{2+} concentrations used in these experiments. This ensured that all assays were performed at the same pH, and obviated pH-induced alterations in ionized Ca^{2+} concentrations.

Protein purification

Proteins were purified from human placenta, spleen or liver using a modification of the method of Haigler *et al.* (1987) as previously described (Ernst *et al.*, 1989). Initial identification of individual annexins was based upon amino acid sequence determinations and/or recognition by specific antibodies, as previously described (Ernst *et al.*, 1989). After purification methods were optimized, individual proteins could be reliably identified by their characteristic behaviour on ion-exchange columns as well as their migration during SDS/PAGE.

Binding of proteins to phospholipids

Liposomes were prepared by hydrating 4 mg of phospholipid (previously dried from chloroform solution under N_2) with 0.5 ml of buffer A. The suspensions were vortex-mixed and sonicated twice. To select liposomes that pelleted readily under the conditions of the assay, pellets were collected from three rounds of centrifugation at 13000 *g*. Final pellets were suspended in 0.25 ml of buffer A. Because neither PA nor PE readily formed stable liposomes when used as pure phospholipids, PA/PE (1:1) and PE/PC (5:1) liposomes were used to measure binding of proteins to PA and PE respectively.

Liposomes (approx. 250 nmol in 20 μl), protein (1.5 μg) and 10 μl of Ca^{2+} stock solutions (final volume of 40 μl) were incubated for 10 min at room temperature, and then centrifuged at 13000 *g* for 15 min. Supernatants and pellets were subjected separately to SDS/PAGE. The gels were stained with Coomassie Blue and destained, and the quantity of protein in each pellet and supernatant was determined using a Quick Quant III densitometer (Helena Laboratories, Beaumont, TX, U.S.A.). The fraction of protein bound to phospholipid (%) was calculated using the integrated area under each peak and the following equation: [pellet/(pellet + supernatant)] × 100. The Ca^{2+} concentration required for half-maximal binding was extrapolated from curves generated from the binding data. Each point on the binding curves represents a value obtained from at least three separate determinations for each Ca^{2+} concentration examined.

Aggregation assay

Liposomes and Ca^{2+} stock solution (final dilution 1:4) were adjusted with buffer A to yield an absorbance at 450 nm between 0.6 and 0.7 (approx. 500 nmol of phospholipid) in a final volume of 0.8 ml. Following a 2 min equilibration, protein was added (140 nM final concentration), and absorbance at 450 nm was monitored continuously for 5 min. Aggregation was verified by phase-contrast microscopy. In order to make comparisons between proteins, results are expressed relative to the change in absorbance observed after 5 min when lipocortin I was incubated with PS liposomes and 450 μM-Ca^{2+}. Because a 10–15% day-to-day variation was observed, lipocortin I standards were run daily as a control.

Membrane fusion assay

Large unilamellar vesicles (LUV) were prepared by reverse-phase evaporation (Düzgünes *et al.*, 1981, 1983). After evaporation, they were extruded through 0.2 μm filters and then 0.1 μm filters to ensure uniform size. Two populations of LUV were prepared, one composed of pure PS and the other containing 98% PS and 1% each of the fluorescent-labelled phospholipids NBD-PE and Rh-PE. The fusion assay was performed at a final phospholipid concentration of 50 mM with a ratio of 9:1 unlabelled to labelled LUV. The final protein concentration was 70 nM in a total volume of 2 ml.

LUV/protein mixtures were excited at 450 nm and emission was monitored continuously at 530 nm on a Spex Fluorolog 2 spectrofluorimeter with a Datamate data acquisition system after the addition of Ca^{2+}, as described previously (Düzgünes *et al.*, 1987).

RESULTS

Preliminary experiments demonstrated that the relative abundance of individual annexins varied considerably in the tissues we examined. For these studies, lipocortin I and calpactin I heavy chain were purified from human placenta and spleen. Lipocortin III was purified from spleen and protein II was from liver. Endonexin II and

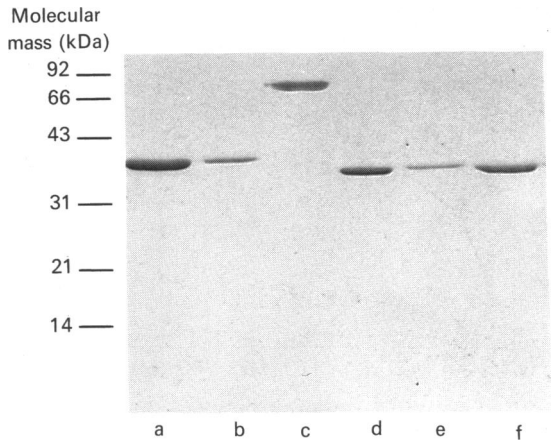

Fig. 1. SDS/PAGE (15% acrylamide) of representative preparations of annexins used in these studies

Proteins were stained with Coomassie Blue. a, Calpactin I monomer; b, lipocortin I; c, p68; d, protein II; e, endonexin II; f, lipocortin III. The positions of molecular mass markers are shown on the left.

p68 were purified from all three tissues. When a protein was purified from more than one tissue, the tissue source conferred no apparent difference in activity in our assays. A representative gel of the purified proteins used in these experiments is shown in Fig. 1. The absence of the calpactin I heterotetramer was confirmed by behaviour of the 36 kDa heavy chain during gel filtration, as well as by the absence of the 10 kDa light chain on SDS/PAGE in samples that were overloaded with 36 kDa heavy chain (18% acrylamide).

Ca^{2+}-dependent binding of annexins to phospholipids

Liposomes used in the phospholipid binding assay were preselected, by repeated centrifugation, to pellet at 13000 *g*. The conditions of the assay were selected to maintain phospholipid in excess, as determined in preliminary studies. Controls included reaction mixtures lacking either Ca^{2+} or phospholipid, in which negligible amounts of proteins were detected in the pellets.

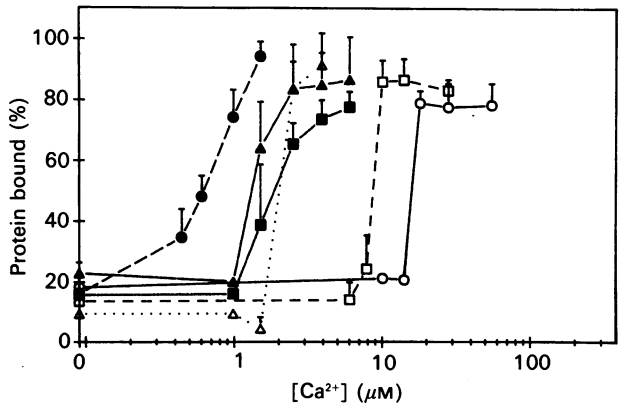

Fig. 2. Representative curves depicting the effects of Ca^{2+} concentration on binding of annexins to PS liposomes

●, Calpactin I heavy chain monomer; ▲, lipocortin I; ■, protein II; △, p68; □, lipocortin III; ○, endonexin II. Each point represents the mean + S.D. calculated from the results of at least three separate experiments.

Table 1. Free Ca^{2+} concentrations for half-maximal phospholipid binding by annexins

Ca^{2+} concentrations (μM) that promote half-maximal binding of annexins to phospholipids were determined by extrapolation from curves plotted using at least three experimental determinations per data point. NB indicates that no binding was detected at ionized Ca^{2+} concentrations up to 370 μM.

	[Ca^{2+}] (μM)				
Protein Phospholipid...	PA	PS	PI	PE	PC
Calpactin I heavy chain	0.2	0.65	1.3	NB	NB
Lipocortin I	0.6	1.3	2.0	NB	NB
p68	0.55	2.0	34	33	NB
Protein II	0.95	2.4	4.4	5.2	NB
Lipocortin III	1.2	8.8	89	170	NB
Endonexin II	1.2	16	130	270	NB

The annexins are known to bind to PS in the presence of Ca^{2+}. Using our assay, we confirmed that all six annexins bound to PS liposomes in a Ca^{2+}-dependent manner. However, we found that the Ca^{2+} concentrations required for half-maximal binding to PS liposomes differed for individual proteins (Fig. 2). Half-maximal Ca^{2+} concentrations required for binding ranged between 0.7 μM for calpactin I heavy chain and 16 μM for endonexin II (Table 1). None of the proteins bound to liposomes composed of PC.

Because none of the annexins bound to PC liposomes, and because we were unable to form stable liposomes using pure PE, binding of annexins to PE was determined using liposomes prepared with a mixture of PE and PC (5:1). Both p68 and protein II bound to liposomes composed of PE/PC; however, they required higher Ca^{2+} concentrations than for binding to PS (Table 1). Although lipocortin III and endonexin II also bound to PE/PC liposomes, they required two orders of magnitude higher Ca^{2+} concentrations than did protein II. Neither calpactin I monomer nor lipocortin I bound to PE at Ca^{2+} concentrations up to 370 μM.

As we observed with PE, PA did not readily form stable liposomes. To examine binding of annexins to PA, PA/PE (1:1) liposomes were used. The Ca^{2+} concentration required to promote half-maximal binding to PA/PE liposomes was less than 2 μM for all six annexins (Table 1). Calpactin I heavy chain, lipocortin I and p68 bound to PA/PE liposomes in the presence of less than 1.0 μM-Ca^{2+}. More than 80% of calpactin I heavy chain bound to PA/PE at 0.44 μM-Ca^{2+}, the lowest Ca^{2+} concentration examined. However, binding of calpactin I heavy chain was not observed in the absence of Ca^{2+}. The 32–33 kDa annexins (protein II, endonexin II and lipocortin III) required between 1 and 2 μM-Ca^{2+} for half-maximal binding to PA/PE liposomes. Because the Ca^{2+} requirements for binding to PA/PE liposomes were significantly less than those required for PE, we feel that these observations reflect the Ca^{2+} concentrations required for binding to PA.

All six annexins bound to PI liposomes. However, binding to PI required higher Ca^{2+} concentrations than those required for binding to either PS or PA (Table 1). Calpactin I heavy chain, lipocortin I and protein II bound to PI at low micromolar Ca^{2+} concentrations (1.3, 2.0 and 4.4 μM respectively). Lipocortin III, p68 and endonexin II required an order of magnitude higher Ca^{2+} concentration for binding to PI compared with binding to PS. Lipocortin III and endonexin II bound at 88 and 130 μM-Ca^{2+} respectively, and p68 bound at 34 μM-Ca^{2+}.

Aggregation of phospholipid vesicles

Liposomes used in the aggregation assay were prepared and selected in a manner identical to those used in the binding assay. No aggregation of liposomes was observed in the absence of protein at Ca^{2+} concentrations up to 1.0 mM, nor did any of the proteins promote aggregation in the absence of Ca^{2+}. To make comparisons between proteins, aggregation data are expressed relative to the change in absorbance observed after 5 min when lipocortin I (140 nM) was incubated with liposomes in the presence of 450 μM-Ca^{2+}. Each protein was assayed at the same molar concentration as was used for lipocortin I. A 2-fold increase in lipocortin I concentration resulted in only a 20% increase in aggregation.

Both calpactin I heavy chain and lipocortin I promoted aggregation of PS liposomes in a Ca^{2+}-dependent manner. An increase in absorbance at 450 nm, which corresponded to aggregation of liposomes as determined by phase-contrast microscopy, was observed with calpactin I heavy chain at Ca^{2+} concentrations as low as 0.98 μM, whereas lipocortin I required 1.5 μM-Ca^{2+} (Fig. 3). With increasing Ca^{2+} concentrations, calpactin I heavy chain (Fig. 4) and lipocortin I increased both the initial rate as well as the extent of aggregation. More than 80% of maximal aggregation was observed in the presence of less than 6 μM-Ca^{2+} for both proteins. A relative steady state (i.e. change in absorbance at 450 nm of less than 0.005 units/min) was reached within 5 min at all Ca^{2+} concentrations examined. Although minor changes in absorbance at 450 nm (less than 10–15% over baseline) were occasionally observed with the other annexins, no aggregation could be verified by phase-contrast microscopy at Ca^{2+} concentrations up to 450 μM.

As we observed with PS liposomes, both calpactin I heavy chain and lipocortin I promoted the aggregation of PI liposomes, although the threshold Ca^{2+} concentrations were somewhat higher (3.9 and 10 μM-Ca^{2+} respectively). Similarly, increased Ca^{2+} concentrations resulted in increases in both the rate and extent of liposome aggregation. However, whereas the total

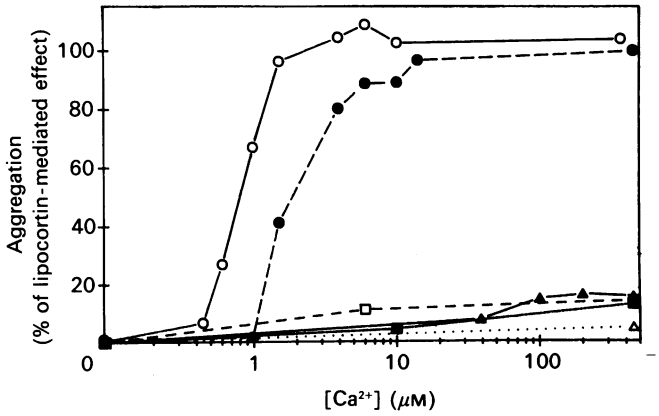

Fig. 3. Effect of Ca^{2+} concentration on annexin-induced aggregation of liposomes

Aggregation of PS liposomes by monomeric annexins. ○, calpactin I heavy chain; ●, lipocortin I; ■, protein II; □, p68; △, lipocortin III; ▲, endonexin II. Results represent the change in absorbance of liposome suspensions after 5 min of incubation and are expressed relative to the change in absorbance at 450 nm observed after 5 min when PS liposomes were incubated with lipocortin I in the presence of 450 μM-Ca^{2+}. The results shown are representative of 2–6 independent experiments. In order to confirm the lack of activity of endonexin II and protein II, assays were performed with protein from two separate preparations.

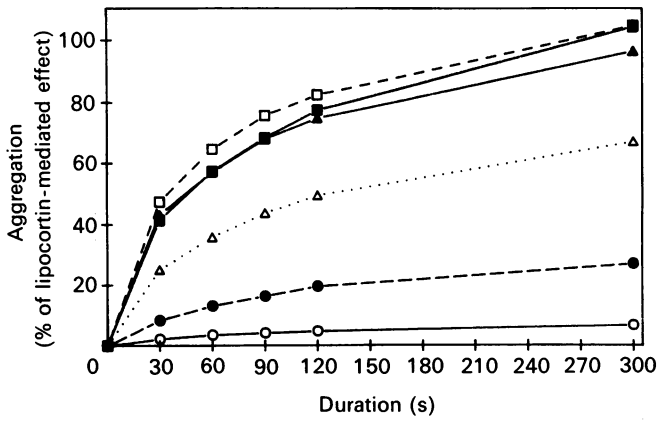

Fig. 4. Effect of Ca^{2+} concentration on the rate and extent of calpactin I heavy-chain-induced aggregation of PS liposomes

Ionized Ca^{2+} concentrations used ranged from 0.44 μM to 450 μM: ○, 0.44 μM; ●, 0.60 μM; △, 0.98 μM; ▲, 1.5 μM; □, 3.9 μM; ■, 450 μM. Aggregation (manifest as change in absorbance at 450 nm) was monitored continuously.

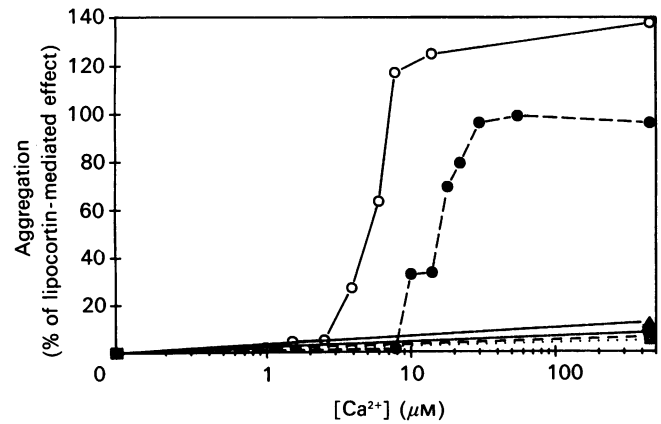

Fig. 5. Annexin-induced aggregation of PI liposomes: effect of Ca^{2+} concentration

○, Calpactin I heavy chain; ●, lipocortin I; ■, protein II; □, p68; △, lipocortin III; ▲, endonexin II. Data were analysed and are expressed as in Fig. 3.

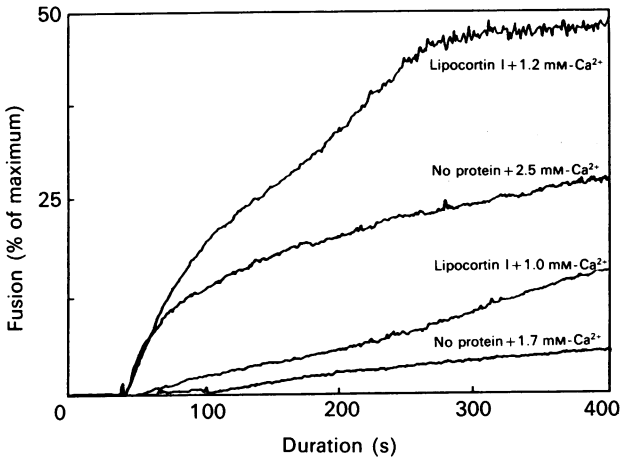

Fig. 6. Effect of lipocortin I on Ca^{2+}-induced fusion of PS-containing LUV

Excitation was at 450 nm and emission was measured at 530 nm. Before initiation of fusion by injection of Ca^{2+}, emission was monitored for 20–30 s to assure stability of the baseline.

change in absorbance at 450 nm promoted by lipocortin I was similar with both PS and PI liposomes, the total change in absorbance that occurred in the presence of calpactin I heavy chain with PI liposomes was 25–30% greater than that observed with PS liposomes (Fig. 5). Annexins which did not promote the aggregation of PS liposomes also did not promote the aggregation of PI liposomes.

Fusion of LUV membranes

In the absence of protein, PS LUV spontaneously fuse in the presence of 2–3 mM-Ca^{2+}. Lipocortin I (70 nM) decreased the threshold Ca^{2+} concentration required for fusion of PS LUV to 1.0 mM (Fig. 6). The threshold for lipocortin I-mediated fusion could be decreased further

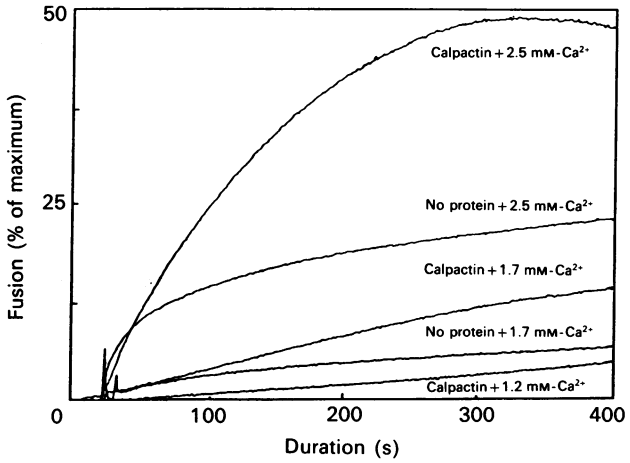

Fig. 7. Effect of calpactin I heavy chain monomer on the rate and extent of Ca^{2+}-induced spontaneous fusion of PS-containing LUV

Experimental procedures were identical with those described in Fig. 6.

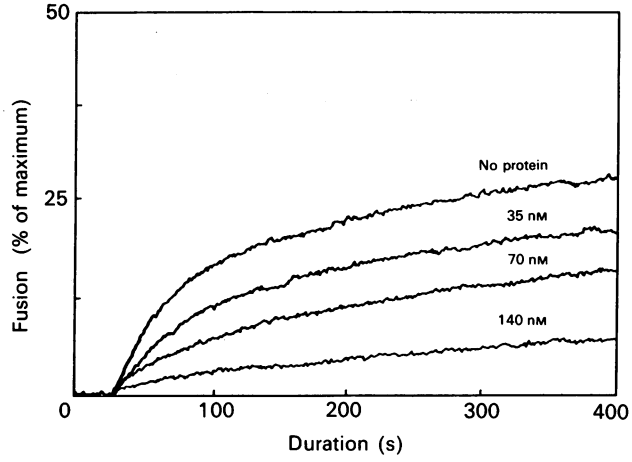

Fig. 8. Inhibition of Ca^{2+}-induced spontaneous fusion of PS-containing LUV by increasing concentrations of p68

All assays were performed in the presence of 2.5 mM-Ca^{2+}.

to 700 μM-Ca^{2+} in the presence of 1.5 mM-Mg^{2+}, whereas Mg^{2+} alone had no effects in this assay. Increases in Ca^{2+} concentrations above threshold resulted in increases in both the rate and extent of LUV fusion, in either the presence or the absence of Mg^{2+}.

Unlike lipocortin I, calpactin I heavy chain did not decrease the Ca^{2+} concentration required for fusion of PS LUV. Although there was no change in the fusion threshold, calpactin I heavy chain did increase the rate and extent of spontaneous fusion at threshold Ca^{2+} concentrations (Fig. 7). In contrast with the enhancement of spontaneous fusion induced by calpactin I heavy chain, p68 inhibited spontaneous fusion at threshold Ca^{2+} concentrations in a concentration-dependent manner (Fig. 8). None of the other annexins exerted detectable effects on fusion of PS LUV under the conditions of our assay.

DISCUSSION

We purified six members of the annexin protein family from human tissues and compared their phospholipid binding specificities as well as their ability to promote aggregation and fusion of phospholipid vesicles. We found that, although these proteins are structurally similar and share several physical properties, individual members of the family exhibit distinct characteristics.

All six of the annexins examined bound PS, PA and PI in a Ca^{2+}-dependent manner. Each protein bound to PA at lower concentrations of Ca^{2+} than required for binding to the other phospholipids. Binding to PS required intermediate Ca^{2+} concentrations, and binding to PI required the highest Ca^{2+} concentrations. Although all six proteins followed this general pattern, the Ca^{2+} requirements for binding to phospholipids varied considerably for individual proteins. For example, half-maximal binding of calpactin I heavy chain to PS required less than 1.0 μM ionized Ca^{2+}, whereas half-maximal binding of endonexin II to PS required 16 μM-Ca^{2+}. Calpactin I heavy chain and lipocortin I exhibited the lowest Ca^{2+} requirements of any of the proteins for binding to PA, PS and PI, but in contrast with the other proteins, they did not bind to PE at any Ca^{2+} con-

centration examined. It is interesting to note that of these proteins, protein II exhibited the narrowest range of Ca^{2+} requirements for binding to PA, PS, PI and PE: there was only a 5-fold difference between the Ca^{2+} concentration required for binding to PA compared with that to PE (0.95 and 5.2 μM respectively). In contrast, lipocortin III exhibited a 100-fold difference between the concentration of Ca^{2+} required for binding to PA compared with PE (1.2 versus 170 μM). As has been reported by others, we found that none of these annexins bound to PC at any Ca^{2+} concentration examined.

In addition to the quantitative differences exhibited by individual annexins with respect to binding to phospholipids, we found a marked qualitative difference when we examined the ability of these proteins to promote aggregation of phospholipid (PS or PI) vesicles. Of the proteins examined, only calpactin I heavy chain and lipocortin I exhibited this activity. Moreover, both of these proteins aggregated PS vesicles at concentrations of ionized Ca^{2+} nearly identical with that required by these proteins for binding to these vesicles, although the maximum rate and extent of vesicle aggregation was observed at higher Ca^{2+} concentrations than those required for maximum binding. Our observation that only two of the annexins we examined promote vesicle aggregation under conditions in which all of these proteins bind to vesicles emphasizes that this activity may relate to a specialized function of lipocortin I and calpactin I.

Three of the annexins we examined influenced fusion of membranes of large unilamellar vesicles prepared with PS. No effects on fusion were observed at concentrations of Ca^{2+} sufficient to promote binding (of all six) or aggregation (lipocortin I and calpactin I). When these proteins were examined in the fusion assay at higher Ca^{2+} concentrations (sufficient to cause slow fusion of LUV in the absence of added protein), we found that three proteins influenced fusion of vesicles, each in a different manner. Lipocortin I reduced the Ca^{2+} concentration required to induce detectable fusion, and in addition augmented the rate and final extent of fusion. Calpactin I heavy chain had no effect on the Ca^{2+} requirement for fusion. However, calpactin I heavy chain did increase the rate and extent of fusion at threshold Ca^{2+} concentrations. In contrast with the effect of lipocortin I and calpactin I, p68 inhibited fusion in this assay. These results suggest that the other annexins serve functions that do not depend on their ability to promote the interaction of two distinct membranes. Alternatively, these other proteins may possess this ability, but require membrane compositions that differ from those which we used in these studies. Further studies will be necessary to determine whether the effects we have observed relate to true biological functions of these proteins.

In summary, we have found that individual members of the annexin family of proteins exhibit differences in phospholipid binding, vesicle aggregation and vesicle membrane fusion. These results imply that individual annexins, despite their similarities, perform distinct intracellular functions. Whereas further investigation is needed to reveal the functions of these proteins *in vivo*, it is likely that all members of the annexin family do not have the same function. Further work is also required to reveal the structural basis for the distinct properties we have observed.

We thank Elaine Hoye for outstanding technical assistance, Dr. Keelung Hong for assistance with the fusion assay and Dr. Ira Goldstein for critical review of the manuscript. This work was supported by grants from the National Institutes of Health (GM11909, AI23697 and AR20684), the Arthritis Foundation, Northern California Chapter and the Robert Wood Johnson Foundation (grant no. 12940).

REFERENCES

Bers, D. M. (1982) Am. J. Physiol. **242**, C404–C408
Brugge, J. S. (1986) Cell **46**, 149–150
Burgoyne, R. D. (1988) Nature (London) **331**, 20
Burns, A. L., Magendzo, K., Shirvan, A., Srivastava, M., Rojas, E., Alijani, M. R. & Pollard, H. B. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 3798–3802
Creutz, C. E., Pazoles, C. J. & Pollard, H. B. (1979) J. Biol. Chem. **254**, 553–558
Crompton, M. R., Owens, R. J., Totty, N. F., Moss, S. E., Waterfield, M D. & Crumpton, M. J. (1988*a*) EMBO J. **7**, 21–27
Crompton, M. R., Moss, S. E. & Crumpton, M. J. (1988*b*) Cell **55**, 1–3
Drust, D. S. & Creutz, C. E. (1988) Nature (London) **331**, 88–91
Düzgünes, N., Wilschut, J., Fraley, R. & Papahadjopoulos, D. (1981) Biochim. Biophys. Acta **642**, 182–195
Düzgünes, N., Wilschut, J., Hong, K., Fraley, R., Perry, C., Friend, D. S., James, T. L. & Papahadjopoulos, D. (1983) Biochim. Biophys. Acta **732**, 289–299
Düzgünes, N., Allen, T. M., Fedor, J. & Papahadjopoulos, D. (1987) Biochemistry **26**, 8435–8442
Ernst, J. D., Hoye, E. & Blackwood, R. A. (1989) Biochem. Biophys. Res. Commun. **161**, 959–964
Geisow, M. J., Walker, J. H., Boustead, C. & Taylor, W. (1987) Biosci. Rep. **7**, 289–298
Glenney, J. R. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 4258–4262
Haigler, H. T., Schlaepfer, D. D. & Burgess, W. H. (1987) J. Biol. Chem. **262**, 6921–6930
Haigler, H. T., Fitch, J. M., Jones, J. M. & Schlaepfer, D. D. (1989) Trends Biochem. Sci. **14**, 48–50
Hong, K., Düzgünes, N., Ekerdt, R. & Papahadjopoulos, D. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 4642–4644
Huang, K.-S., Wallner, B. P., Mattaliano, R. J., Tizard, R., Burne, C., Frey, A., Hession, C., McGrey, P., Sinclair, L. K., Chow, E. P., Browning, J. L., Ramachandran, K. L., Tang, J., Smart, J. E. & Pepinsky, R. B. (1986) Cell **46**, 191–199
Klee, C. (1988) Biochemistry **27**, 6645–6653
Meers, P., Ernst, J. D., Hong, K., Düzgünes, N., Goldstein, I. M. & Papahadjopoulos, D. (1987) J. Biol. Chem. **262**, 7850–7858
Pepinsky, R. B., Tizard, R., Mattalino, R. J., Sinclair, L. K., Miller, G. T., Browning, J. L., Chow, P. E., Burne, C., Huang, K., Pratt, D., Wachter, L., Hession, C., Frey, A. Z. & Wallner, B. P. (1988) J. Biol. Chem. **263**, 10799–10811
Saris, C. J. M., Tack, B. F., Kristensen, T., Glenney, J. R. & Hunter, T. (1986) Cell **46**, 201–212
Südhof, T. C., Slaughter, C. A., Leznicki, I., Barjon, P. & Reynolds, G. A. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 664–668
Wallner, B. P., Mattaliano, R. J., Hession, C., Cate, R. L., Tizard, R., Sinclair, L. K., Foeller, C., Chow, E. P., Browning, J. L., Ramachandron, K. L. & Pepinsky, R. B. (1986) Nature (London) **320**, 77–81
Weber, K., Johnson, N., Plessman, U., Von, P. N., Soling, H.-D., Ampe, C. & Vandekerckove, J. (1987) EMBO J. **6**, 1599–1604

Received 11 August 1989/9 October 1989; accepted 23 October 1989