Regulation of the Chromaffin Granule Aggregating Activity of Annexin I by Phosphorylation†

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Received June 3, 1992; Revised Manuscript Received August 6, 1992

ABSTRACT: Annexin I (lipocortin I) binds to secretory granule membranes and promotes their aggregation in a Ca^{2+}-dependent manner [Creutz, C. E., et al. (1987) *J. Biol. Chem. 262*, 1860–1868; Drust, D. S., & Creutz, C. E. (1988) *Nature 331*, 88–91]. It is also phosphorylated on serine residues when bovine chromaffin cells are stimulated to secrete [Michener, M. L., et al. (1986) *J. Biol. Chem. 261*, 6548–6555], suggesting phosphorylation may be involved in modulating the function of annexin I. We report here that phosphorylation of the N-terminal tail by protein kinase C strongly inhibits the ability of annexin I to aggregate chromaffin granules by increasing the calcium requirement 4-fold. This inhibition was readily reversed when the protein was dephosphorylated by protein phosphatase 2A. The inhibition was not due to inability of phosphorylated annexin I to bind to chromaffin granules, since the phosphorylated form bound to the granule membrane at slightly lower levels of calcium than the native form. The phosphorylated annexin I also bound to 20% phosphatidylserine/80% phosphatidylcholine vesicles at lower Ca^{2+} levels than the native form. The inhibitory effect of phosphorylation on the granule aggregating activity of annexin I was found to be amplified by an unusual mechanism: The phosphorylated form inhibited the activity of the unphosphorylated form. The possible importance of the regulation of annexin I activity by phosphorylation in exocytosis is discussed.

Annexin I (lipocortin I) is a member of the annexin family of Ca^{2+}-dependent membrane-binding proteins [for reviews, see Geisow and Walker (1986), Klee (1988) and Burgoyne and Geisow (1989)]. These proteins possess four or eight conserved 70 amino acid repeats which comprise the so-called core domain. The core domain harbors the binding sites for Ca^{2+} and phospholipid. Each annexin also has an N-terminal tail which is variable in length and sequence.

Annexin I is a substrate for protein kinase C and for the epidermal growth factor receptor kinase in vitro and in vivo (Summers & Creutz, 1985; Michener et al., 1986; Fava & Cohen, 1984; Sawyer & Cohen, 1985). Both the tyrosine and the serine/threonine phosphorylation sites have been identified in the N-terminal tail of human annexin I (Haigler et al., 1987; Schlaepfer et al., 1988). Although no biological function of the annexins has been firmly established, the ability of certain annexins to promote contact between membranes in a Ca^{2+}-dependent manner has prompted the hypothesis that these proteins may be involved in membrane trafficking during exocytosis and phosphorylation might be a way to modulate their functions (Drust & Creutz, 1988; Ali et al., 1989; Nakata et al., 1990; Oshry et al., 1991). In fact, annexin I is phosphorylated on serine residues in stimulated chromaffin cells (Michener et al., 1986). It has also been shown that tyrosine phosphorylation decreases the Ca^{2+} requirement of human annexin I for binding to phosphatidylserine vesicles (Schlaeper & Haigler, 1987). We report here that phosphorylation by protein kinase C strongly *inhibits* the ability of bovine annexin I to aggregate chromaffin granules while mildly *promoting* the binding of annexin I to the granule membrane.

EXPERIMENTAL PROCEDURES

Materials. Phospholipids were purchased from Avanti Polar Lipids (Birmingham, AL). [γ-^{32}P]ATP was from New England Nuclear (Boston, MA). Sephadex G-25 columns were obtained from Pharmacia.

Annexin Preparations. Annexins I and II were purified from bovine lung tissue according to Haigler et al. (1987) with modifications. Lung tissue (200 g) was homogenized in a Waring blender for 1 min with 2 volumes (w/v) of buffer H: 20 mM Hepes (pH 7.4 at 4 °C), 150 mM KCl with aprotinin (0.12 trypsin inhibitor unit/mL), 1 μg/mL leupeptin, 1 mM (PMSF),[1] 0.5 mM DTT, 1 mM benzamidine, and 4 mM iodoacetic acid. The homogenate was centrifuged at 2900*g* for 15 min. The pellet was reextracted with another 2 volumes of buffer H and then homogenized for 10 s at full speed with a Brinkmann Polytron PT3000 tissue grinder. This homogenate was centrifuged at 2900*g* for 15 min, and the two supernatants were combined. $CaCl_2$ was added to the combined supernatant to 2 mM final concentration. After standing on ice for 20 min, the extract was centrifuged at 100000*g* for 30 min. The pellet was resuspended in 40 mL of buffer H with 1 mM $CaCl_2$ and centrifuged again at 100000*g* for 30 min. This wash step was repeated two more times. The final pellet was resuspended in buffer H minus iodoacetic acid but containing 5 mM EGTA. The extract was centrifuged at 130000*g* for 1 h. The supernatant was dialyzed overnight, with three changes of buffer, against 20 mM Tris (pH 8.65 at 4 °C), 0.1 mM PMSF, 0.5 mM DTT, and 0.5 mM EGTA and chromatographed on a DEAE-Sephacel column (1.6 ×

† This work was supported by NIH Grants CA40042 and DK33151.

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[1] Abbreviations: PMSF, phenylmethanesulfonyl fluoride; DTT, dithiothreitol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; MES, 2-[*N*-morpholino]ethanesulfonic acid; Hepes, *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]; PS, phosphatidylserine; PC, phosphatidylcholine; PKC, protein kinase C; PP2A, protein phosphatase 2A; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

0006-2960/92/0431-9934$03.00/0

15 cm). The column was eluted with linear gradients of ammonium acetate, pH 8.65, of the following volume and concentrations: 150 mL of 0–200 mM; 100 mL of 200–500 mM. The flow rate was 0.5 mL/min, and 2.5-mL fractions were collected. Two protein peaks, eluting at 30 and 150 mM ammonium acetate, contained annexin I and II, respectively. They were collected separately and adjusted to pH 6.0 before being applied to an FPLC Mono S column (1 mL) equilibrated in 25 mM MES (pH 6.0 at 4 °C). A linear gradient of 0–500 mM KCL in 25 mM MES was applied. Annexin I eluted at 130 mM KCl, and annexin II eluted at 320 mM KCl. Using these purification methods, 5 mg of annexin I and 10 mg of annexin II were routinely purified from 200 g of lung tissue. The preparations were estimated to be more than 95% pure by SDS–PAGE.

Annexin IV was purified from bovine liver according to Creutz et al. (1987).

Preparation of Chromaffin Granules, Granule Membranes, and Phospholipid Vesicles. Chromaffin granules were prepared by differential centrifugation in 0.3 M sucrose as described by Pazoles and Pollard (1978). Granule membranes were prepared by the method of Bartlett and Smith (1974). Multilamellar liposomes were prepared by mixing chloroform solutions of phospholipids in various ratios, dried under argon, and resuspended by vortex mixing into 20 mM Hepes, pH 7.0 at 22 °C.

Phosphorylation of Annexins. Protein kinase C, purified from rat brain, was a generous gift from Richard Burack and Julianne Sando (University of Virginia). The phosphorylation reaction was carried out in a mixture containing 5 μg of annexin, 20 mM Hepes, pH 7.4, at 22 °C), 0.3 mM $CaCl_2$, 5 mM $MgCl_2$, 0.1% mercaptoethanol, 100 μM [γ-^{32}P]ATP (10 mCi/mol), and 0.1 mM total lipid with 60% phosphatidylserine, 35% phosphatidylcholine, and 5% diacylglycerol. The reaction was initiated by adding the kinase. After 60 min at room temperature, the reaction was terminated by adding EGTA to a final concentration of 2 mM. The mixture was then centrifuged at 100000*g*, at 4 °C, for 1 h to spin down the lipid. The supernatant was applied to a Pharmacia G-25 column to separate the protein from salts and free ATP. The stoichiometry of phosphorylation was 1.04 ± 0.21 mol of phosphate per mole of annexin I (n = 7). Phosphorylated annexin I was used in the aggregation assay without further separation. Annexins II and IV were phosphorylated to much lower stoichiometry (0.2 and 0.05 mol of phosphate per mole of annexin II and IV, respectively) and could not be used directly in the aggregation assay.

The phosphorylation of annexin I occurred entirely in the N-terminus, as demonstrated by cleavage of the N-terminus with plasmin (Huang et al., 1987) and examination of the tail and core domains by SDS–PAGE and autoradiography. Phosphorylated annexin IV was treated with trypsin (Weber et al., 1987) to confirm that the phosphorylation site was located at the N-terminus.

Dephosphorylation of Annexins. Protein phosphatase 2A (PP2A), purified from bovine heart, was a generous gift from Dr. Timothy Haystead (University of Virginia). The dephosphorylation reaction was carried out at 22 °C for 30 min in a buffer containing 40 mM Hepes (pH 7.0 at 22 °C), 30 mM KCl, 240 mM sucrose, and 1 mM DTT.

Binding Assays. Five micrograms of annexins was preincubated with chromaffin granule membranes or multilamellar PS/PC liposomes for 15 min at 22 °C in 100 μL of buffer containing 20 mM Hepes (pH 7.0), 30 mM KCl, and Ca^{2+}-EGTA buffer to give a final concentration of 2.5 mM EGTA and the desired free Ca^{2+} concentration (checked with a Ca^{2+}-selective electrode, Radiometer, Copenhagen, Denmark). The mixture was centrifuged for 1 h at 100000*g* in a Beckman TL-100 table-top ultracentrifuge. Both the supernatants and the pellets were run on 10% SDS–polyacrylamide gels according to Laemmli (1970). An excess amount of lipid (0.5 mM) and granule membranes (50 μg of protein/mL) was used in the assay. Binding experiments without membranes were done in the presence of 0.8 mg/mL ovalbumin as carrier protein to prevent nonspecific binding.

Aggregation Assays. Chromaffin granule aggregation was assayed by measuring the change in turbidity (absorbance at 540 nm) as described (Creutz et al., 1978) at room temperature in a buffer containing 40 mM Hepes (pH 7.0), 30 mM KCl, and 240 mM sucrose. Absorbance measurements were made on a Beckman DU70 microprocessor-controlled spectrophotometer equipped with an automatic cuvette positioner which allowed intermittent monitoring of six simultaneous reactions. After establishing an absorbance baseline, aggregation was initiated by adding 20 μL of Ca^{2+}-EGTA buffer with hand mixing, and absorbance was recorded for an additional 8 min. Protein concentration was measured by the method of Bradford (1976). Protein titrations were done to determine the amount of annexins required for maximal levels of aggregation. Either phosphorylated or unphosphorylated annexin I (4 μg/mL) gave maximal aggregation at high calcium level (2.5 mM), and this concentration was used for all Ca^{2+} titrations of granule aggregation.

Statistical Analysis. Membrane binding data, shown in Figures 1 and 2, were analyzed using a Kolmogorov–Smirnov one sample test (Conover, 1980; Gibbons, 1971; software used was the KSONE procedure of the IMSL STAT/LIBRARY, version 1.1, 1989, from IMSL, Houston, Texas). In each case, the data from three independent experiments were combined. The differences between the data for the phosphorylated and unphosphorylated forms were compared with a Gaussian distribution with the same standard deviation centered at 0. The value of P given in each case represents the probability that the parent distribution is also centered at 0. That is, P represents the probability that the titration curves for the phosphorylated and unphosphorylated forms are the same.

RESULTS

Effect of Phosphorylation on the Binding of Annexin I to Phospholipid Vesicles. In the presence of greater than 10 μM Ca^{2+}, annexin I specifically binds acidic phospholipids, such as phosphatidylserine. Although a previous study of tyrosine phosphorylation of annexin I was done with pure PS vesicles (Schlaepfer & Haigler, 1987), we decided to use a lower, more physiological, composition of acidic phospholipid vesicles to examine the effect of phosphorylation by protein kinase C. The mixture of native annexin I and ^{32}P-labeled annexin I was incubated with 20% PS/80% PC vesicles in the presence of increasing free Ca^{2+} concentrations. The vesicles were then subjected to centrifugation, and both supernatants and pellets were fractionated by SDS–PAGE. The native protein was quantitated by Coomassie Blue staining and two-dimensional densitometry. The ^{32}P-labeled protein was quantitated either by autoradiography and densitometry or by Cerenkov counting of gel slices. Both methods gave similar results. In these experiments, the ^{32}P-labeled protein was present in trace amount (<5%) in the mixture and was an insignificant fraction of the total Coomassie Blue-stained band.

As shown in Figure 1, phosphorylation by protein kinase C enhanced the Ca^{2+} sensitivity of annexin I to bind

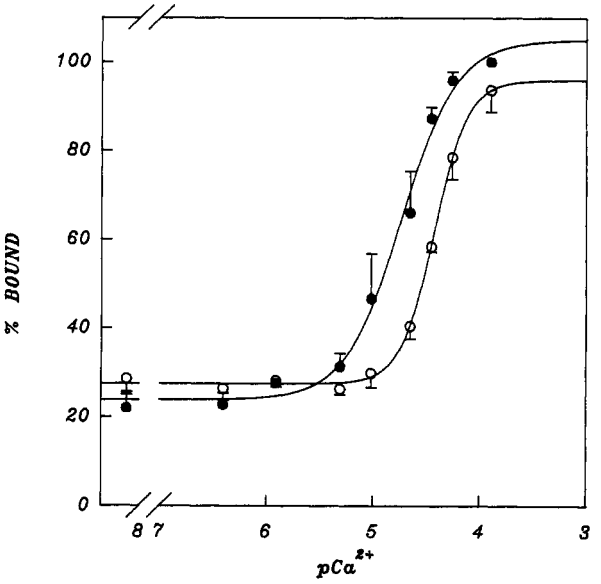

FIGURE 1: Binding of annexin I to 20% phosphatidylserine/80% phosphatidylcholine vesicles. (○) Unphosphorylated annexin I; (●) phosphorylated annexin I. The graph represents data (mean and SEM) averaged from three independent experiments. The curves were compared using a Kolmogorov–Smirnov one sample test as described under Experimental Procedures. The *P* value, representing the probabilities that the titration curves of the phosphorylated and unphosphorylated forms are the same, is <0.006.

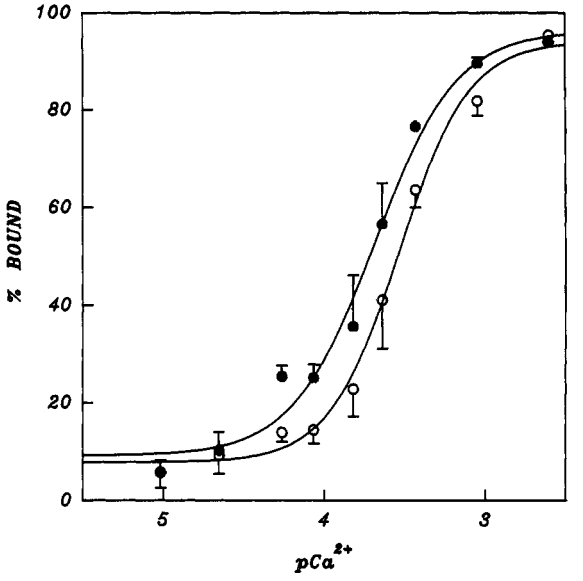

FIGURE 2: Annexin I binding to chromaffin granule membranes. (○) Unphosphorylated form; (●) phosphorylated form. The data are the average of three independent experiments. A value of $P < 0.001$ was obtained by the Kolmogorov–Smirnov test applied to the two sets of data (see Experimental Procedures).

phospholipid vesicles. The Ca^{2+} required for half-maximal binding was 9.0 μM for the phosphorylated form versus 21.3 μM for the native protein. When similar experiments were performed on annexin II (calpactin tetramer) and annexin IV (endonexin I), we also saw a small difference between the phosphorylated and unphosphorylated forms. The half-maximal Ca^{2+} concentration was 0.93 μM for the unphosphorylated annexin II, 0.60 μM for the phosphorylated annexin II ($p < 0.007$), 3.35 μM for the unphosphorylated annexin IV, and 4.72 μM for the phosphorylated annexin IV ($p < 0.123$).

Effect of Phosphorylation on the Binding of Annexin I to Chromaffin Granule Membranes. Since annexin I has been shown to associate with chromaffin granule membranes (Creutz et al., 1987) and promote granule aggregation (Drust & Creutz, 1988), we sought to determine the effect of phosphorylation of annexin I on its binding to chromaffin granule membranes. As shown in Figure 2, phosphorylation caused a small shift in the Ca^{2+} titration curve for binding to lower levels of Ca^{2+}. The Ca^{2+} required for half-maximal binding was around 206 μM for the phosphorylated form and 292 μM for the native form. The lower sensitivities to calcium in the case of binding to granule membranes, compared to binding to lipid vesicles, may reflect the low percentage of acidic phospholipids in the granule membranes (Winkler & Carmichael, 1982).

Effect of Phosphorylation of Annexin I on the Aggregation of Chromaffin Granules. Although phosphorylation did not greatly affect the binding of annexin I to chromaffin granule membranes, we tested whether phosphorylation might directly modulate the granule aggregating activity of the protein. As shown in Figures 3a and 4, unphosphorylated annexin I aggregated chromaffin granules with half-maximal Ca^{2+} at 174 μM, similar to a previous report (Drust & Creutz, 1988). Phosphorylation by protein kinase C, however, inhibited the granule aggregation by annexin I, particularly at submaximal levels of calcium (Figures 3b and 4). The half-maximal Ca^{2+} concentration for aggregation by the phosphorylated form was shifted to 682 μM. This inhibition of annexin I to aggregate granules by phosphorylation could not be simply attributed to an inhibition of granule membrane binding (see Figure 2). It is also unlikely that the inhibition was caused by protein denaturation during the phosphorylation procedure because, as shown in Figure 3c, when the phosphorylated annexin I was incubated with protein phosphatase 2A (PP2A) for 30 min prior to the aggregation assay, this inhibition was readily reversed. PP2a dephosphorylated annexin I to completion after 30 min of incubation (Figure 3c, inset). In the presence of 1 μM okadaic acid, which inhibited PP2A activity, annexin I remained phosphorylated, and no appreciable granule aggregation was seen below $pCa^{2+} = 3.632$ (Figure 3d). PP2A itself did not have granule-aggregating activity even at millimolar calcium concentration (Figure 3e).

The degree of inhibition of granule aggregation depended on the extent of phosphorylation. When annexin I was phosphorylated to lower stoichiometry (0.34 mol of phosphate incorporated/mole of protein), the half-maximal Ca^{2+} concentration for granule aggregation was shifted to only 316 μM, instead of 682 μM as in the stoichiometrical (1:1) phosphorylation.

Phosphorylated Annexin I Acted as an Inhibitor of the Unphosphorylated Protein. To further investigate the interaction between the phosphorylated and the unphosphorylated annexin I, we mixed the two forms at different ratios (Figure 5). At $pCa^{2+} = 3.632$, which was approximately the half-maximal Ca^{2+} concentration for the unphosphorylated form, the granule-aggregating activity of the mixture decreased with increasing amount of phosphorylated annexin I. When 4 μg of unphosphorylated annexin I, which was a saturating amount of protein for the aggregation of granules under the conditions of the assay, was mixed with 4 μg of phosphorylated form, the aggregating activity was decreased by more than 80%. Since both the unphosphorylated and the phosphorylated proteins bound to granule membranes with similar Ca^{2+} sensitivities (Figure 2), this result suggested that the phosphorylated form might inhibit the unphosphorylated form by competing for binding sites on the granule.

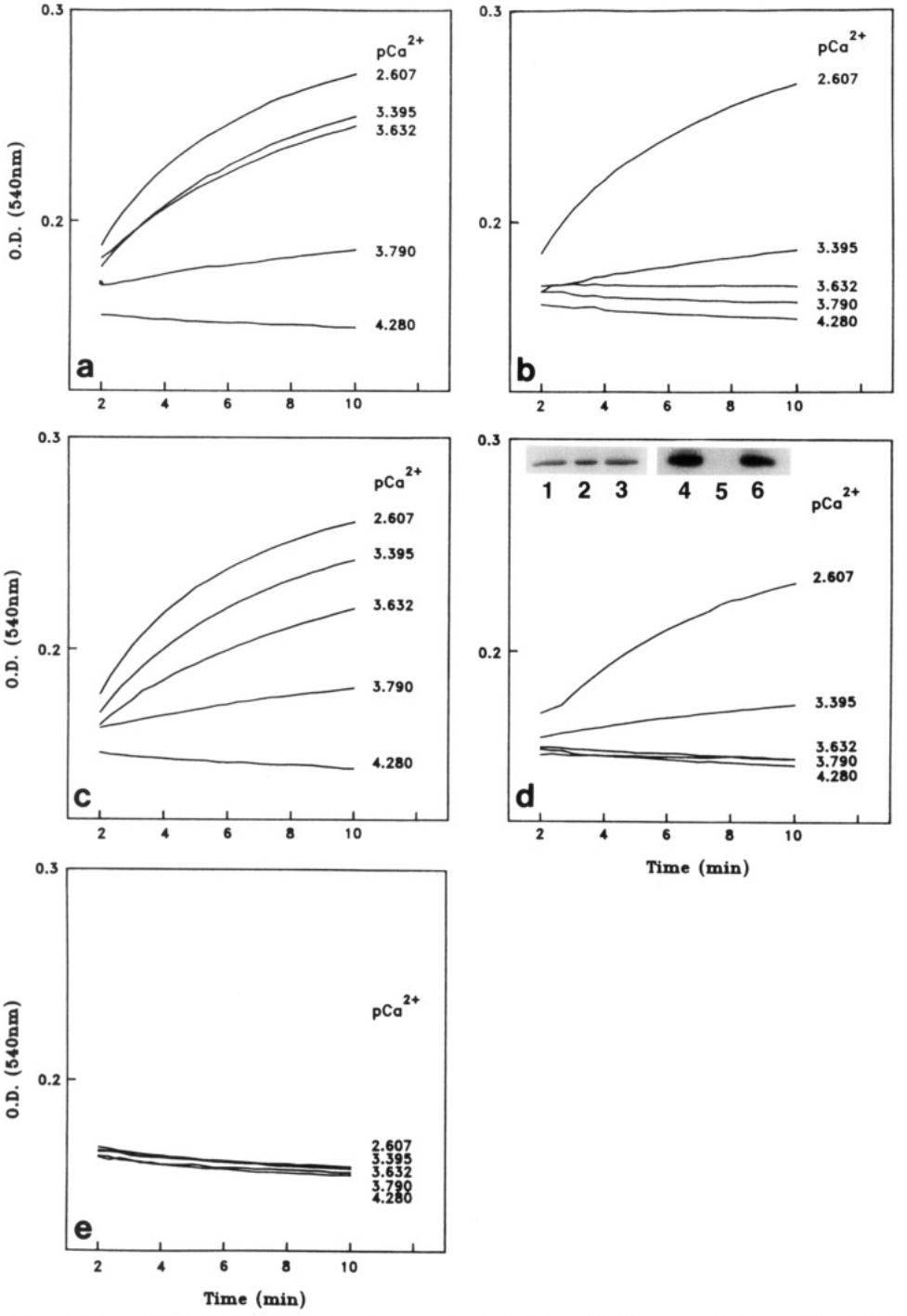

FIGURE 3: Time course of chromaffin granule aggregation by annexin I at different Ca^{2+} concentrations. The initial absorbance of the chromaffin granule suspension was approximately 0.15 at 540 nm. Four micrograms of either unphosphorylated or phosphorylated annexin I was added. Concentrations were determined to give maximal levels of aggregation at 2.5 mM Ca^{2+}. The aggregation was initiated by adding Ca^{2+}-EGTA buffer with the indicated free Ca^{2+} concentrations. The absorbance was monitored for 8 min at 540 nm. (a) Unphosphorylated annexin I, 4.0 μg; (b) Phosphorylated annexin I, 4.0 μg; 4.0 μg of phosphorylated annexin I incubated with phosphatase 2A (PP2A) in the absence (c) or presence (d) of 1 μM okadaic acid for 30 min before addition to the granules. PP2A only was also tested (e). Inset: (1 and 4) phosphorylated annexin I; (2 and 5) phosphorylated annexin I incubated with PP2A for 30 min; (3 and 6) phosphorylated annexin I incubated with PP2A in the presence of okadaic acid. (1–3) Coomassie blue stain; (4–6) autoradiograph.

DISCUSSION

We report here that phosphorylation on the N-terminus of bovine annexin I inhibits its ability to aggregate chromaffin granules at low levels of Ca^{2+}. Recombinant human annexin I expressed in yeast was also found to behave the same way (data not shown). This inhibition occurs even though the phosphorylation slightly enhances the binding of annexin I to granule membranes at low levels of Ca^{2+}. This paradoxical result suggests that additional mechanisms, besides initial membrane binding, are involved in promoting membrane-membrane contacts. These additional mechanisms might involve annexin–annexin interactions between membranes (Creutz & Sterner, 1985; Zaks & Creutz, 1991) or activation of a second membrane-binding site on an already membrane-bound annexin. The initial membrane binding and the subsequent membrane aggregation phenomena might be dependent on structural features on opposite surfaces of the protein.

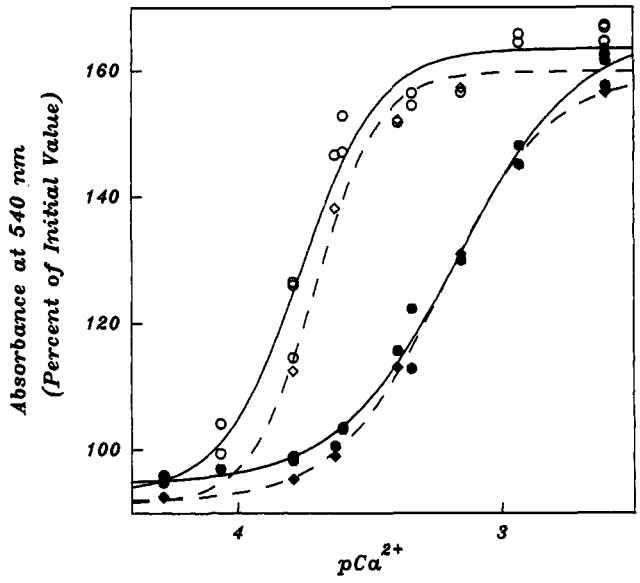

FIGURE 4: Ca^{2+}-titration curves for chromaffin granule aggregation by annexin I: unphosphorylated (○); phosphorylated (●); phosphorylated incubated with PP2A in the absence (◇) or presence of okadaic acid (◆). The absorbance at 540 nm was expressed as percent of initial value. In the absence of aggregating agents, there was a gradual decline in turbidity due to granule lysis, which resulted in final turbidity values less than 100%. The continuous lines represent the best fit of the expression $y = m(kx)^n [1 + (kx)^n] + c$ to the data (m, maximum; k, constant; n, Hill coefficient; c, constant). Solid lines, no phosphatase; dashed lines, in the presence of PP2A.

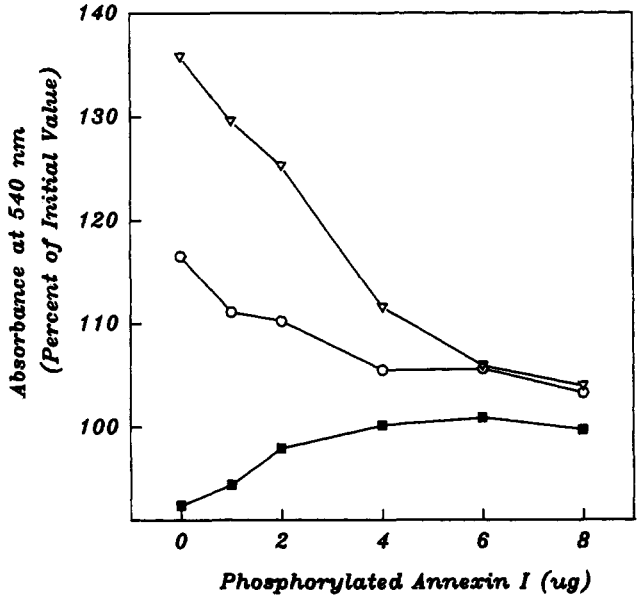

FIGURE 5: Phosphorylated annexin I inhibits the ability of unphosphorylated annexin I to aggregate chromaffin granules. Granule aggregation was assayed at $pCa^{2+} = 3.632$. (■) 0 μg, (○) 2 μg, or (▽) 4 μg of unphosphorylated annexin I was mixed with increasing amounts of phosphorylated annexin I and added to the granule suspension. The increase in turbidity (A_{540}) in 8 min is plotted as a function of the amount of phosphorylated annexin I in the mixture. The data are representative of three independent experiments using two separate preparations of annexin I and chromaffin granules.

The X-ray crystal structure of annexin V (Huber et al., 1990a,b) has shown that the four tandem repeats of the protein are folded into four domains of similar structure. Each consists of five α-helices wound into a right-handed superhelix. The four domains are arranged in a planar array, with the Ca^{2+}-binding sites located on a slightly convex surface, which presumably also binds phospholipid membranes. The N-terminus, however, is located at the concave surface of the molecule, opposite from the membrane attachment site. The high degree of sequence identity (>40%) among members of the annexin family suggests that other annexins may have similar structures. The membrane–membrane aggregation event may therefore involve the "cytoplasmic" face of the membrane-bound molecule and the N-terminal tail.

The mechanism of the regulation of the chromaffin granule aggregating activity of annexin I by phosphorylation has a particularly unusual and effective feature not frequently seen in the control of enzyme activity by phosphorylation: Not only is the aggregating activity inhibited by phosphorylation but the phosphorylated form of the protein becomes an inhibitor of the active form. As shown in Figure 5, when 4 μg of unphosphorylated annexin I, which was the saturating amount of protein to aggregate granules at $pCa^{2+} = 3.632$, was mixed with equal amount of the phosphorylated form, granule aggregation was greatly decreased. This might be explained by competition for binding sites on the membrane because both forms bind granule membranes with similar Ca^{2+} sensitivities.

At higher calcium concentrations ($pCa^{2+} = 2.6$), phosphorylated annexin I aggregated granules to the same extent as the unphosphorylated form. A different mechanism may be involved in promoting membrane aggregation at this high level of calcium. Such a phenomenon has been observed with annexin IV (Zaks & Creutz, 1991). At low levels of Ca^{2+}, the aggregation of membranes by annexin IV is correlated with the formation of contacts between annexin IV molecules on different membranes. At high calcium concentrations, no intermembrane self-association of annexin IV molecules was observed while extensive membrane aggregation still occurred. Annexin IV might undergo a conformational change or might interact with the membrane in a different orientation, at high Ca^{2+} concentrations, to allow an annexin monomer to simultaneously bind two membrane surfaces at once. By analogy, the unphosphorylated annexin I might aggregate membranes through the formation of annexin–annexin contacts between membranes at low levels of calcium; at high levels of calcium, the phosphorylated protein might be unable to form annexin–annexin contacts but be able to act as a monomer to bring two membranes together.

The annexin II tetramer (calpactin I) promotes chromaffin granule aggregation at submicromolar Ca^{2+} concentrations (Drust & Creutz, 1988). This heterotetramer is composed of two annexin-like molecules of a "p36" heavy chain connected together by binding to a "p10" light chain dimer at the N-terminal 12 residues of the heavy chains. This structure might be viewed as a preformed protein complex ready to bind two membranes. Although p36 and its N-terminal truncated form, p33, bind Ca^{2+} and membranes with similar affinities (Glenney, 1986), p36 is unable to promote granule aggregation at low levels of Ca^{2+}, while p33 can (Drust & Creutz, 1988). This further supports the notion that the annexin N-terminus is more critically involved in membrane–membrane aggregation than in membrane binding. The N-terminus is inhibitory in the case of the calpactin heavy chain, and the inhibition is relieved by proteolysis or light chain binding. In the case of annexin I, the N-terminus becomes inhibitory when it is phosphorylated by protein kinase C.

The Ca^{2+} levels required for annexin I to bind and aggregate chromaffin granules are rather high, compared to average intracellular free calcium concentrations ($[Ca^{2+}]_i$). However, it is important to realize that Ca^{2+} may be compartmentalized in the cell and exist in rather high local concentrations near

Ca^{2+} channels upon stimulation. A recent report (Llinas et al., 1992), using a low-sensitivity calcium-dependent photoprotein (n-aequorin-J) injected into the presynaptic terminal of the giant squid synapse, showed that the $[Ca^{2+}]_i$ reached 200–300 μM at secretory sites during stimulation. $[Ca^{2+}]_i$ at sites of secretion in single adrenal chromaffin cells has also been inferred to be 10 μM or greater by measuring rates of secretion during depolarization (Augustine & Neher, 1992).

Annexin I promotes chromaffin granule contacts in vitro (Drust & Creutz, 1988), similar to those seen between granules during compound exocytosis in the chromaffin cell. It also promotes the fusion of liposomes with the plasma membranes of neutrophils in vitro, a system which has been proposed as a model for membrane fusion in exocytosis (Oshry et al., 1991). If the membrane-aggregating activity of this protein is indeed required for membrane fusion in exocytosis, then the observed phosphorylation of annexin I in stimulated chromaffin cells (Michener et al., 1986) may play a role in downregulating exocytosis. In addition, dephosphorylation of annexin I by a specific phosphatase may be necessary to initiate exocytosis.

ACKNOWLEDGMENT

We are grateful to Ruth Stornetta for assistance with the 2-D densitometry, Lindsay Faunt and Michael Johnson for the statistical analysis, Richard Burack and Julianne Sando for protein kinase C, and Timothy Haystead for phosphatase 2A and okadaic acid.

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