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Endogenous cleavage of annexin I generates a truncated protein with a reduced calcium requirement for binding to neutrophil secretory vesicles and plasma membrane

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Abstract

We have earlier shown that an N-terminal truncated annexin I molecule, annexin I_{des1-8}, is generated in human neutrophils through cleavage by a membrane localized metalloprotease. The truncated protein showed differences in membrane binding among the neutrophil granule populations as compared to full-length annexin I. In this study, we investigated the cleavage capabilities of isolated neutrophil secretory vesicles and plasma membrane, and the binding of full-length annexin I and annexin I_{des1-8} to these membrane fractions. Translocations were performed in vitro to secretory vesicles and plasma membrane, respectively, at different Ca^{2+} concentrations. We show that the annexin I-cleaving membrane localized metalloprotease is present both in the secretory vesicles and the plasma membrane. The N-terminal truncation of annexin I gives rise to a molecule with a decreased Ca^{2+} requirement for binding, both to secretory vesicles and plasma membrane. There was, thus, no difference in binding of either full-length annexin I or annexin I_{des1-8} to the secretory vesicles as compared to the plasma membrane.

Keywords: Annexin I; Cleavage; Ca^{2+} requirement; Neutrophil secretory vesicle; Plasma membrane

1. Introduction

Neutrophil exocytosis is a process of outermost importance for our defense against infections induced by invading microbes. The protection is mediated by a release of antimicrobial proteins, stored in the subcellular granules of the phagocyte, into phagosomes or to the extracellular milieu. The secretory process is dependent on increased levels of intracellular Ca^{2+} as well as on translocation of various cytosolic proteins to the granule membranes and/or to the plasma membrane [1–5]. Calcium-regulated, phospholipid-binding proteins, such as the annexins, are thus candidate regulators in exocytosis processes, and many members of this family have been extensively studied in different cellular systems [1,5–9]. All members of the annexin family contain a conserved calcium- and phospholipid binding domain of 70 amino acids, repeated four or eight times [10]. The N-terminal parts of the annexins differ both with respect to length and amino acid composition. Most probably these differences confer the functional individuality of the family members. Among the annexins, annexin I has gained most attention for being

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involved in the regulation of exocytosis. In addition to its membrane binding qualities, this molecule also possesses the capacity to aggregate membrane vesicles and mediate membrane fusion [1,6,7,11–14].

Our previous studies have shown that annexin I binds calcium dependently to all the different neutrophil membrane compartments investigated, i.e. the azurophil granules, the specific granules, and a mixed light membrane fraction containing the secretory vesicles and the plasma membrane [15]. Furthermore, neutrophils contain an annexin I-cleaving protease, and the truncated annexin I protein binds selectively and calcium dependently to specific granules and secretory vesicles/plasma membrane, but not to azurophil granules [15,16]. We have characterized the protease as well as the truncated annexin I molecule [17]. These studies show that cleavage is mediated by a metalloprotease present in the membrane of specific granules and secretory vesicles/plasma membrane, but absent from the azurophil granule membranes. Amino acid sequencing show that the annexin I molecule is cleaved between the amino acids leucine and lysine in positions eight and nine in the N-terminal domain of the protein [17].

The light membrane fraction that we have used in our earlier studies is enriched in both secretory vesicles and plasma membrane. The secretory vesicles of neutrophils are the most easily mobilized organelle and it contains a number of different membrane receptors that are exposed on the cell surface upon cell activation [18]. The fact that the matrix content in this subcellular organelle consists of plasma proteins suggests that it has a unique origin as compared to the granules. Whereas the neutrophil granules (i.e. the azurophil, specific, and gelatinase granules) are derived from the ER–Golgi complex, the secretory vesicles are formed by endocytosis [19,20]. This possibly takes place by internalization of plasma membrane in the maturing neutrophil via a special endocytic pathway that does not result in fusion with lysosomal compartments and degradation of the endocytosed matrix.

Translocation of annexin I to endosomes has in other cell types been shown to critically depended on the N-terminal domain of the protein [21], which made it interesting for use to study translocation of annexin I to neutrophil endosomes as compared to granules. The aim of the present study was to determine the presence/absence of the annexin I-cleaving protease in isolated secretory vesicles and plasma membrane as well as assessing the calcium-dependent binding of full-length annexin I and annexin I_{des1-8} to these membranes.

We found that the annexin I-cleaving protease is present both in the secretory vesicles and in the plasma membrane and that annexin I_{des1-8} has a reduced Ca^{2+} requirement as compared to full-length annexin I when binding to these membranes. We could, however, not detect any difference in binding either of annexin I or annexin I_{des1-8} between secretory vesicles and plasma membrane, respectively.

2. Materials and methods

2.1. Materials

Reagents used in this work were obtained from the following sources. Percoll and Ficoll-Paque were purchased from Pharmacia, Uppsala, Sweden. The adenosine 5′-triphosphate (ATP), 5-bromo-4-chloro-3-indolyl phosphate (BCIP), ethylene glycol-bis-(β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), NitroBlue tetrazolium (NBT), phenylmethanesulfonyl (PMSF), *p*-nitrophenylphosphate and Triton X-100 were products from Sigma, St. Louis, MO. The diisopropyl fluorophosphate (DFP) was obtained from Aldrich, Steinhem, Germany. The dithiothreitol (DTT) was a product from Merck, Darmstadt, Germany. The Pefabloc and bovine serum albumin (BSA) were obtained from Boehringer Mannheim, Mannheim, Germany. The PD-10 Sephadex G-25 M columns were from Pharmacia Biotech AB, Uppsala, Sweden. Ultrafree-CL filters and poly(vinylidene difluoride) (PVDF) membranes were purchased from Millipore, Bedford, MA. Goat anti-mouse IgG was a product from Jackson ImmunoResearch Laboratories, West Grove, PA. Monoclonal mouse anti-annexin I was from Zymed, San Francisco, CA.

2.2. Isolation of granulocytes

Human polymorphonuclear leukocytes were isolated from buffy coats [22]. Erythrocytes were sedimented in dextran and leukocytes were collected

from the supernatant by centrifugation. The remaining erythrocytes were removed by hypotonic lysis. Mononuclear leukocytes were removed by centrifugation on Ficoll-Paque. The isolated granulocytes were washed twice in Krebs–Ringer medium without Ca^{2+} (120 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 1.7 mM KH_2PO_4, 8.3 mM Na_2HPO_4, 10 mM glucose, pH 7.3) and resuspended in cold physiological saline before diisopropyl fluorophosphate treatment (DFP; 0.2 μl/ml cell suspension, 10 min). The cells were finally resuspended in cold relaxation buffer (100 mM KCl, 3 mM NaCl, 3.5 mM $MgCl_2$, 10 mM PIPES, pH 7.4) supplemented with 1 mM $ATP(Na)_2$, 0.5 mM PMSF, and 1 mM Pefabloc

2.3. Disruption of cells and isolation of cytosol and subcellular organelles

The cytosol and the subcellular organelles were isolated according to the method described by Borregaard et al. [23]. Neutrophils in relaxation buffer were disrupted by nitrogen cavitation (2500 kPa, 5 min; Parr, Moline, IL). EGTA (1.5 mM) was added to the homogenate to avoid aggregation of granules. The homogenate was centrifuged at $400\times g$ for 15 min (4°C) and the post-nuclear supernatant was collected. To separate azurophil granules, specific granules and secretory vesicles/plasma membrane, the post-nuclear supernatant was layered on top of a two-layer Percoll gradient with densities of 1.05 and 1.12 g/ml. After centrifugation at $32\,800\times g$ (35 min, 4°C) the three different bands containing subcellular organelles were collected from the top of the gradient by aspiration with a Pasteur pipette. The bands characterized by marker protein analysis are denoted α (azurophil granules, myeloperoxidase as marker), β (specific granules, vitamin B_{12} as marker) and γ (secretory vesicles/plasma membrane, alkaline phosphatase as marker) in order of decreasing density.

Secretory vesicles (γ1) and plasma membrane (γ2) were separated by centrifugation on a floatation gradient of Percoll [24]. The post-nuclear supernatant was mixed with an equal volume of the heavy Percoll solution (1.12 g/ml) and placed in the middle of the gradient between Percoll of densities 1.04 and 1.12 g/ml. After centrifugation, four bands were visible and the gradient was fractionated from the bottom of the tub. The localization of organelles in the fractions was established by marker analysis. Alkaline phosphatase was measured as the cleavage of *p*-nitrophenyl phosphate (2 mg/ml) in a 1 mM $MgCl_2$, 50 mM sodium barbital buffer (pH 10.5) in the presence or absence of Triton X-100 (0.4%) [25]. The secretory vesicle enriched fraction (γ1), was contaminated to about 20% with plasma membrane, and the same goes for the purity of the plasma membrane enriched fraction (γ2).

Cytosol was isolated from a post-nuclear supernatant, prepared without EGTA, by centrifugation to remove organelles ($100\,000\times g$, 60 min at 4°C). Translocation of cytosolic proteins to secretory vesicles and plasma membrane was performed as described earlier [16].

2.4. Calcium-induced cleavage of annexin I

Cytosol and secretory vesicles/plasma membrane were mixed in a ratio of 1:3. Calcium chloride (2 mM) was added and the sample was incubated at 37°C to induce cleavage. The reaction was stopped after 30 min by the addition of EGTA (4 mM). Samples were mixed 1:1 in sample buffer (0.0625 M Tris–HCl, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.001% bromophenol blue) and stored at −20°C until analyzed.

2.5. Inhibition of annexin I cleavage by dithiothreitol

Cytosol was mixed with secretory vesicles/plasma membrane in a ratio of 1:3. Dithiothreitol (DTT; 5 mM) and Ca^{2+} (2 mM) were added and the sample was incubated for 30 min at 37°C. The reaction was stopped by adding EGTA (4 mM) and the samples were stored in reduced sample buffer at −20°C until analyzed.

2.6. Preparation of full-length annexin I and annexin I_{des1-8}

Cytosol was mixed with secretory vesicles/plasma membrane in a ratio of 1:3 and Ca^{2+} (2 mM) was added. The sample was incubated for 30–45 min to induce cleavage and stopped by the addition of EGTA (4 mM) and then centrifuged ($100\,000\times g$, 30 min., at 4°C). This treatment results in a sample

with approximately 50% full-length annexin I and 50% annexin I_{des1-8}, but we have only used samples in which the amount of the full-length annexin I molecule is equal or in excess of the truncated molecule.

The buffer was changed to relaxation buffer by using a PD-10 Sephadex G-25 M column. The sample was concentrated by centrifugation in Ultrafree-CL filters (low binding cellulose, 10 000 NMWL) and stored on ice until used.

2.7. Calcium-induced translocation of full-length annexin I and annexin I_{des1-8} to secretory vesicles and plasma membrane at different calcium concentrations

The light membrane fraction containing secretory vesicles and plasma membrane or isolated secretory vesicles and plasma membrane, respectively, were resuspended in relaxation buffer supplemented with Pefabloc (1 mM) and mixed with the pre-cleaved cytosol in a ratio of 3:1. In order to inhibit further cleavage of full-length annexin I, DTT (5 mM) was added. Different concentrations of Ca^{2+} (i.e. 1, 0.6, 0.3, 0.1, 0.05, and 0.01 mM) were added and the mixtures were incubated at 37°C for 15 min to induce translocation of annexin I to the organelles. After centrifugation in an air fuge (Beckman Instruments, Fullerton, CA) at 20 000 psi for 15 min, the collected membranes were washed once in relaxation buffer containing DTT (5 mM), Pefabloc (1 mM), and Ca^{2+} of the same concentration as in the first step. To release the translocated annexins, the membranes were resuspended in relaxation buffer supplemented with EGTA (2 mM) and Pefabloc (1 mM) and incubated at 37°C for 15 min. After centrifugation at 20 000 psi for 20 min the supernatants were collected and stored in sample buffer at −20°C until analyzed.

2.8. SDS–PAGE and immunoblotting

Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) in 10% polyacrylamide gels [26] under reduced conditions and transferred electrophoretically to PVDF membranes [27]. The PVDF-membranes were incubated in blocking buffer (1% BSA, 1% skim milk, 0.05% Tween-20 in PBS) for 1 h in room temperature and then for at least 1 h with monoclonal mouse anti-annexin I antibodies (1:5000 dilution). The membranes were finally incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (1:2000 dilution). The blots were developed with BCIP (0.15 mg/ml) and NBT (0.3 mg/ml) in 0.1 M $NaHCO_3$, 1 mM $MgCl_2$, pH 9.8.

3. Results

3.1. Generation of annexin I_{des1-8} from full-length annexin I in neutrophil cytosol

Annexin I binds to neutrophil subcellular organelles in a calcium-dependent manner (shown for the light membrane fraction containing secretory vesicles and plasma membrane in Fig. 1). This translocation is accompanied by cleavage of the molecule illustrated by the appearance of a 39-kDa band in addition to the 40-kDa full-length band (Fig. 1). We have earlier shown that the truncated form of annexin I lacks eight amino acids at the N-terminal part of the protein and is accordingly designated annexin I_{des1-8}. In order to be able to compare binding of the full-length annexin I and annexin I_{des1-8}, without accompanying cleavage of annexin I, we exploited the fact the proteolytic activity of the annexin I-cleaving protease is totally inhibited by DTT [17] (Fig. 2). A mixture of annexin I and annexin I_{des1-8} was prepared by incubating the light membrane with cytosol

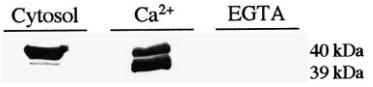

Fig. 1. Translocation of annexin I from neutrophil cytosol to secretory vesicles/plasma membrane. Secretory vesicles/plasma membrane isolated by Percoll gradient centrifugation were incubated with cytosol and Ca^{2+} (2 mM). The membranes were collected by centrifugation and washed. Annexin I was released from the membrane by extraction in an EGTA-containing buffer. Following centrifugation the supernatant was electrophoresed under reducing conditions, electroblotted onto PVDF membranes and detected with mouse anti-annexin I antibodies. The cytosol contained only full-length annexin I (40 kDa, left). Full-length annexin I and annexin I_{des1-8} (40 and 39 kDa) were detected following translocation to secretory vesicles/plasma membrane in the presence of Ca^{2+} (middle), but not in the presence of EGTA (right).

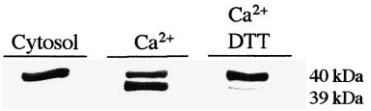

Fig. 2. Cleavage of annexin I from neutrophil cytosol by secretory vesicles/plasma membrane. Cytosol (left) was incubated with secretory vesicles/plasma membrane in the presence of Ca^{2+} (2 mM), with or without DTT (5 mM), for 30 min at 37°C. The samples were electrophoresed under reducing conditions and electroblotted onto PVDF-membranes and detected with mouse anti-annexin I antibodies. Annexin I was Ca^{2+}-dependently cleaved (Ca^{2+}, middle). By adding DTT (right) the cleavage was inhibited.

and Ca^{2+} (2 mM final concentration) for 30 min at 37°C. The reaction was terminated by the addition of EGTA (4 mM final concentration) and the membrane fraction was removed by centrifugation. After buffer exchange, DTT (5 mM) was added and the sample harboring both the full-length annexin I as well as the annexin I_{des1-8}, in fairly equal amounts, could be used to determine differences in membrane binding between the two proteins.

3.2. Ca^{2+}-induced binding of full-length annexin I and annexin I_{des1-8} to the light membrane fraction

To determine if there is any difference in binding properties between the two annexin I molecules, calcium-induced translocation experiments were performed, using the pre-cleaved DTT containing cytosol and the light membrane fraction (i.e. secretory vesicles/plasma membrane). Translocation experiments were performed at different Ca^{2+} concentrations (1, 0.6, 0.3, 0.05 and 0.01 mM). We found that the truncated form of annexin I needs less calcium for binding to these membranes compared with full-length annexin I (Fig. 3). At a calcium concentration of 50 μM, full-length annexin I retains no calcium-dependent membrane binding, while annexin I_{des1-8} still remains bound to the membrane.

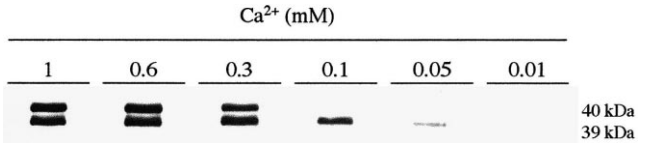

Fig. 3. Translocation of full-length annexin I and annexin I_{des1-8} from neutrophil cytosol (right) to secretory vesicles/plasma membrane. Isolated secretory vesicles/plasma membrane were mixed with cytosol in the presence of DTT (5 mM) at different Ca^{2+} concentrations. The membranes were collected and washed. Full-length annexin I and annexin I_{des1-8} were released from the membranes by extraction in an EGTA-containing buffer. The organelles were removed and the supernatants were electrophoresed under reducing conditions, electroblotted onto PVDF-membranes and detected with mouse anti-annexin I antibodies. The translocations show that full-length annexin I needs more Ca^{2+} for membrane binding than compared with annexin I_{des1-8}. At 0.05 mM Ca^{2+}, annexin I_{des1-8} is capable of binding, while full-length annexin I is incapable of binding.

3.3. Separation of secretory vesicles and plasma membrane

Neutrophil post-nuclear material was fractionated on a modified Percoll gradient in which the light membranes were separated by floatation. The gradient disclosed two light-membrane bands, and based on the distribution profiles for alkaline phosphatase activity (marker for the plasma membrane) and latent alkaline phosphatase activity (marker for the secretory vesicles), we conclude that the modified gradient permits a clear separation of secretory vesicles (γ1; Fig. 4) from the plasma membrane

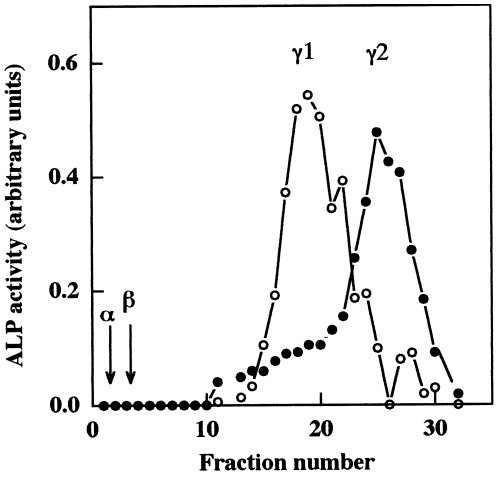

Fig. 4. Marker analysis by alkaline phosphatase (ALP) activity in neutrophil subcellular fractions. The postnuclear supernatant of disrupted neutrophils was fractionated on a floatation Percoll gradient to separate the secretory vesicles from the plasma membrane. Fractions were analyzed for alkaline phosphatase by measuring the cleavage of *p*-nitrophenyl phosphate. Alkaline phosphatase was measured in the absence of Triton X-100 (marker for the plasma membrane; ●) and in the presence of Triton X-100 reflecting the enzyme content in both the plasma membrane and the secretory vesicles. The latent alkaline phosphatase (marker for the secretory vesicles; ○) was calculated as the difference between ALP (+Triton) and ALP (−Triton).

(γ2; Fig. 4). The γ1 and γ2 fractions were used for cleavage experiments and translocation of the annexin I molecules.

3.4. Annexin I cleavage induced during translocation to neutrophil secretory vesicles and plasma membranes

Annexin I binds to neutrophil secretory vesicles and plasma membrane, in a calcium-dependent manner. Translocation to both these membrane fractions is accompanied by the cleavage of annexin I, illustrated by the appearance of a 39-kDa protein in addition to the full-length band (Fig. 5). The protease that cleaves the annexin I molecule is, thus, present both in the plasma membrane and in the secretory vesicle.

3.5. Calcium-induced translocation of full-length annexin I and annexin I_{des1-8} to isolated secretory vesicles and plasma membrane

To investigate the Ca^{2+} requirement for binding of full-length annexin I and annexin I_{des1-8} to organelles with different membrane composition and origin, translocation of the molecules to secretory vesicles and plasma membrane were performed at different Ca^{2+} concentrations (Fig. 6A,B). We used neutrophil cytosol containing a mixture of full-length and truncated annexin I (see above) as source for translocation and the experiments were performed in the presence of DTT in order to inhibit further cleavage of the full-length molecule.

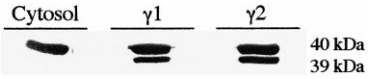

Fig. 5. Cleavage of annexin I by separated secretory vesicles and plasma membrane. Annexin I was mixed with the same amounts (in cell equivalents) of isolated secretory vesicles and plasma membrane, respectively, in the presence of Ca^{2+} (1 mM) and incubated at 37°C for 45 min. Annexin I was released from the membranes by extraction with EGTA (2 mM). The organelles were removed and the supernatants were electrophoresed under reducing conditions and electroblotted onto PVDF-membranes. Annexin I was detected with mouse anti-annexin I antibodies. Full-length annexin I (left) was cleaved Ca^{2+}-dependently by both secretory vesicles (γ1, middle) and plasma membrane (γ2, right) to generate annexin I_{des1-8}.

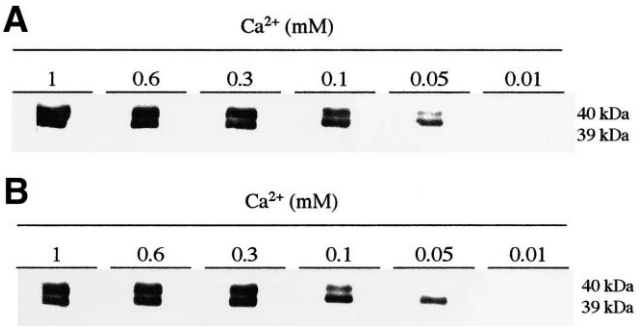

Fig. 6. Translocation of full-length annexin I and annexin I_{des1-8} in neutrophil cytosol to secretory vesicles (A) and plasma membrane (B) at different Ca^{2+} concentrations. The same amounts (in cell equivalents) of secretory vesicles and plasma membrane, respectively, were mixed with cytosol in the presence of Ca^{2+}. The membranes were collected and washed. Annexin I was released from the membranes by adding EGTA (2 mM). The membranes were removed and the supernatants were electrophoresed under reducing conditions, electroblotted onto PVDF-membranes and detected with mouse anti-annexin I antibodies. The cytosol contained both full-length annexin I and annexin I_{des1-8} (right). Following translocation, annexin I_{des1-8} was bound to secretory vesicles and plasma membrane, respectively, at 0.05 mM Ca^{2+}, while full-length annexin I needs a higher Ca^{2+} concentration.

The results of the translocation experiments show that the calcium requirement differs between the two annexin I molecules in that annexin I_{des1-8} requires less calcium for membrane-binding to both secretory vesicles (Fig. 6A) and plasma membrane (Fig. 6B) as compared to the full-length annexin I. Very little or no binding of the full-length protein is obtained at concentrations of Ca^{2+} below 0.3 mM, whereas binding of annexin I_{des1-8} was observed also at a Ca^{2+} concentration of 50 μM. There was no difference, either of annexin I or annexin I_{des1-8}, in binding between secretory vesicles and plasma membrane, respectively.

4. Discussion

A truncated annexin I molecule, annexin I_{des1-8}, is formed during Ca^{2+}-mediated translocation of full-length annexin I to neutrophil specific granules and secretory vesicles/plasma membrane [15,17]. The truncation is carried out by a membrane localized metalloprotease [17], and in agreement with the functional properties of the full-length protein, annexin

I_{des1-8} binds Ca^{2+} dependently to both specific granules and to a light membrane fraction enriched in secretory vesicles/plasma membrane. However, in contrast to the full-length protein, annexin I_{des1-8} does not bind to neutrophil primary (azurophil) granules, placing the function of the N-terminal part of the annexin I molecule in focus. Most studies show that modulation through proteolytic truncation or phosphorylation of serine and/or threonine residues of the N-terminal part of annexin I affects the properties of the molecule [12,14,28–32]. It has been suggested that such modulations may be of importance for fusogenic as well as secretory events in which annexin I has a regulatory function [12,14,21,28–32]. However, the results vary between different studies and the discrepancies might reflect differences between the techniques used to modify the annexin I molecule as well as biological differences between the membranes/subcellular organelles used to determine annexin I binding.

Through the use of a fractionation technique that allows us to separate neutrophil secretory vesicles from the plasma membrane [24] we can show that the neutrophil protease responsible for cleavage of annexin I is present in both these organelles and that Ca^{2+} induces translocation of both full-length annexin I and annexin I_{des1-8} to secretory vesicles as well as to plasma membrane.

The secretory vesicles are probably formed through an internalization of plasma membrane derived endocytic vesicles [19,20]. However, the composition of the membranes in the two compartments differs. For example, the content of HLA class 1 antigen and a number of different receptors have different distribution between the secretory vesicles and the plasma membrane, suggesting that some membrane molecules are sorted out of the endosomes whereas others, such as the annexin I-cleaving metalloprotease, are sorted in (to be excluded or included in the endocytic vesicle, respectively) during formation of the secretory vesicle. In other cell types, the membrane composition of endocytic vesicles may also be changed during processing/maturation of the vesicles. With respect to annexin I binding it has been shown that the translocation of annexin I to early endosomes is critically dependent on a full-length N-terminal domain, whereas association with late endosomes is facilitated by N-terminal truncation of the molecule [21]. The fact that the secretory vesicles are of endocytic origin made it interesting to investigate if there is any difference in the binding of the full-length annexin I and annexin I_{des1-8} to secretory vesicles and plasma membrane, respectively. We could not, however, detect any difference with respect to calcium requirement for binding to the two membranes. Furthermore, binding of annexin I_{des1-8} to these organelles required less Ca^{2+} than the full-length protein. The molecular mechanism behind this phenomenon is not known, but it has been suggested that an N-terminal modifications of annexin I induces conformational changes in the phospholipid binding C-terminal domain that directly enhances its phospholipid binding capacity [14,28,33]. The difference in Ca^{2+} sensitivity between full-length annexin I and annexin I_{des1-8} could, however, be due to different regulatory roles of these proteins in the exocytosis process since it has been suggested that the aggregation and fusion processes are dependent on the interaction of the N-terminal of two annexin I molecules bound to different membranes [10,30,34].

With respect to annexin I binding characteristics of the neutrophils organelles, the secretory vesicles resemble late endosomes whereas the azurophil granules (to which binding is critically dependent on a full-length N-terminal domain of annexin I) resemble early endosomes [21]. The later organelle was earlier classified as the lysosomes of the neutrophil, but the fact that they are devoid of LAMPs (lysosomal associated membrane proteins) [24,35] suggests that they should be re-classified. The functional characteristics of these two organelles differ in that the secretory vesicles contain membrane components that are delivered to the cell surface during cell activation whereas the bactericidal content of the azurophil granules are delivered primarily to a selected part of the plasma membrane (the phagosome) that surrounds ingested bacteria. Whether the different annexin I binding characteristics of these neutrophil organelles might be of importance for regulation of their unique functions, remains to be determined.

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