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Diverse effects of different neutrophil organelles on truncation and membrane-binding characteristics of annexin I

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Abstract

A neutrophil annexin I-related protein, detected after translocation of cytosolic proteins to specific granules and secretory vesicles/plasma membrane (Sjölin et al. (1994) Biochem. J. 300, 325–330), has been characterized with respect to origin and organelle-binding properties. The annexin I-related protein is formed as a result of annexin I cleavage, and this occurs during translocation of annexin I to the specific granules and secretory vesicles/plasma membrane, but not when annexin I is translocated to azurophil granules. The cleavage required calcium and it was facilitated in the presence of specific granules or secretory vesicles/plasma membrane, but not in the presence of azurophil granules. We conclude that the membranes of specific granules and secretory vesicles/plasma membrane contain a protease which is able to cleave annexin I into a truncated 38 kDa fragment, which retains the ability to bind to these organelles. The azurophil granules lack the capacity to cleave annexin I as well as the ability to bind the 38 kDa fragment. These findings may implicate a role for annexin I in the divergent regulation of exocytosis of the different neutrophil granules.

Keywords: Annexin I; Neutrophil organelle; Membrane binding

1. Introduction

Neutrophil granulocytes play a central role as a first line of defence against micro-organisms. After phagocytosis of a microbe, killing and degradation occurs through the action of toxic oxygen metabolites, microbicidal proteins and a variety of proteolytic enzymes. Recognition of the microbes as well as the killing process is dependent on mobilisation of granule constituents. Four different intracellular granules have been described in the neutrophil, and these granules contain a variety of mobilizable receptors and matrix proteins [1]. Mobilization of these different types of granules occurs independently of each other, as is clearly illustrated by the fact that the neutrophil granule subsets need different threshold levels of intracellular calcium in order to be secreted. Secretory vesicles are the organelles most easily mobilized, followed by gelatinase granules and specific granules. Azurophil granules are the least mobilizable ones. Little is at present understood about the regulation of this differential exocytosis, but it is believed to involve proteins that are able to bind the different granules in response to a calcium signal. Such regulatory, granule-binding proteins could work either by binding specifically to each type of granule or the same proteins could interact differently with different granules. Both models could of course also work in concert.

Annexins are a group of Ca^{2+}-regulated, phospholipid-binding proteins, which have been suggested to participate in/regulate granule secretion and phagolysosome fusion [2]. Many annexins are present in the human neutrophil [3–6], and we have earlier shown that several of these can bind to different neutrophil organelles isolated by subcellular fractionation on Percoll gradients [4]. Among those, annexin I proved to bind to azurophil granules, specific granules and secretory vesicles/plasma membrane. However, we also observed that an annexin I-related protein of lower molecular mass (38 kDa compared to 40 kDa for annexin I) showed some type of selectivity in binding, as it was translocated to the specific granules and the secretory vesicles/plasma membranes, but not to the azurophil granules.

Members of the annexin family are characterized by the

Abbreviations: PMSF, phenylmethylsulphonyl fluoride; PVDF, polyvinylidene difluoride.

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ability to bind, above all, negatively charged phospholipids in a calcium-dependent manner. With one exception, annexins are built up of four repeats of a 70 amino acid sequence. The repeats express a high degree of identity with each other and the calcium- and phospholipid-binding sites reside in this part of the molecule [2]. The N-terminal sequences of annexins are unique to each annexin species and structural alterations in this part of the molecule may act to regulate specific functions of the different proteins. Furthermore, many annexin members specifically bind certain proteins in their N-terminal, which in the case of annexin I can be exemplified by binding of actin [7]. The ability of annexin I to aggregate and facilitate fusion of secretory granules [8,9], has implicated a role of annexin I as partaker in the exocytic machinery of cells [10]. Annexin I also mimics the effect of glucocorticoids [11], and is secreted extracellularly by a novel secretory mechanism [12]; which complicates further the understanding of the function(s) of this molecule. Phosphorylation of the N-terminal part of annexin I has been shown to inhibit its membrane-aggregating activity while, at the same time, the calcium requirement for granule/phospholipid binding changes [13]. Proteolytic cleavage in the N-terminal part of annexin I has also been shown to change the biological properties of the molecule, in that less calcium is required for phospholipid binding [14], and the ability to aggregate granules is altered [15].

The aim of this study was to investigate the origin of the neutrophil 38 kDa annexin I-like protein and to characterize its membrane-binding properties using different neutrophil organelles.

2. Materials and methods

2.1. Isolation of granulocytes

Human polymorphonuclear granulocytes (neutrophils) were isolated from buffy coats as described before [4] according to the method of Bøyum [16].

2.2. Isolation of cytosol and organelles

Isolated neutrophils were resuspended in relaxation buffer (100 mM KCl, 3 mM NaCl, 3.5 mM $MgCl_2$, 10 mM Pipes, 1 mM $ATP(Na)_2$, 0.5 mM phenylmethylsulphonyl fluoride (PMSF), pH 7.4) supplemented with Pefabloc (1 mM) and disintegrated by nitrogen cavitation [17]. For preparation of cytosol, the cavitate was collected without EGTA and all organelles were removed by centrifugation at $100\,000 \times g$ for 1.5 h (4°C). The pellet was discarded and the supernatant (cytosol) stored at 4°C until used [4]. For isolation of azurophil granules (α), specific granules (β) and secretory vesicles/plasma membrane (γ), EGTA was added to the nitrogen cavitate. The postnuclear supernatant was centrifuged on a two-step density gradient of Percoll. The α, β, and γ bands were collected and washed free of EGTA and Percoll and thereafter resuspended in relaxation buffer. Each organelle fraction was checked by marker molecule analyses [4] and kept at 4°C until used.

2.3. Translocation of cytosolic proteins

Translocation of cytosolic proteins to azurophil granules (α), specific granules (β) and secretory vesicles/plasma membrane (γ), respectively, was performed as described earlier [4].

2.4. Interaction of annexin I with neutrophil organelles

The standard conditions for studying the effects on annexin I of interaction with neutrophil organelles was as follows. Cytosol and organelles were mixed in ratio 2/1 (i.e. $(2–6) \times 10^7$ cell equivalents of cytosol was mixed with $(1–3) \times 10^7$ cell equivalents of the respective organelle preparation) in Eppendorf tubes and Pefabloc was added to 1 mM. $CaCl_2$ was added to give a final concentration of 2 mM and the samples were incubated in a 37°C water bath for 10–75 min. The incubation was stopped by adding Laemmli sample buffer (reduced) containing 5 mM EGTA. Samples were heat treated (100°C) for 5 min and kept frozen at −20°C until analysed. In some series of experiments the cytosol/organelle ratios were varied.

2.5. Sequential interaction of cytosolic proteins with different neutrophil organelles

Cytosol and organelles of either the α, β or γ fraction was mixed in ratio 2/1 and $CaCl_2$ was added to a final concentration of 2 mM. The mixture was incubated at 37°C for 15 min and thereafter centrifuged at $100\,000 \times g$ for 20 min (4°C). The supernatant (denoted 'remaining cytosol') was collected and processed as described in the next paragraph. The pellet consisting of organelles was washed once and thereafter resuspended in relaxation buffer supplemented with 5 mM EGTA. The organelles were incubated and centrifuged in an airfuge (Beckman Instruments) at $100\,000 \times g$ for 10 min. The supernatant contained the proteins that were associated with the organelle membranes and which, upon calcium chelation, were released from them.

The supernatant, denoted 'remaining cytosol', was used to resuspend another, fresh aliquot of organelles. This mixture was incubated and centrifuged as described in the previous paragraph. The resulting pellet and supernatant were processed accordingly. In this manner, the same aliquot of cytosol was used for up to four incubations with organelles, in the order indicated in the figures.

2.6. SDS / PAGE and immunoblotting

SDS/PAGE was performed according to Laemmli [18] using 10% (T) homogenous polyacrylamide gels which

were electroblotted according to Towbin [19] onto PVDF membranes. Membranes were blocked, then incubated with monoclonal annexin I antibodies as described [4]. Quantification of cleaved product was estimated by densitometry using a flat bed scanner and IPLab gel software (Signal Analytics)

2.7. Chemicals

Monoclonal mouse anti-annexin I was from Zymed Laboratories, San Francisco, CA, USA. ATP and PMSF were bought from Sigma Chemical Co., St. Louis, MO, USA. Percoll was from Pharmacia, Uppsala, Sweden. Pefabloc was from Boehringer-Mannheim, Germany. The PVDF membrane was purchased from Millipore, Bedford, MA, USA.

3. Results

3.1. Translocation of annexin I to neutrophil organelles

Fractionation of neutrophils on discontinuous Percoll gradients isolates four fractions (not shown) which are identified as cytosol, secretory vesicles/plasma membrane (γ), specific granules (β) and azurophil granules (α). The proteins belonging to the annexin family are recovered in the cytosolic fraction. Annexin I was detected using a monoclonal antibody, which binds to a single 40 kDa band on immunoblots of neutrophil cytosol (Fig. 1).

Previous work has shown that many proteins of the annexin family are able to bind to the different types of neutrophil organelles which can be isolated in Percoll gradients [4]. In order to study Ca^{2+}-dependent translocation of annexin I to neutrophil organelles, the α (azurophil granules), β (specific granules) and γ (secretory vesicles/plasma membrane) fractions were isolated and separately suspended in cytosol and the calcium concentration was raised. Following removal of the cytosol, the organelles were washed and resuspended in a buffer containing EGTA, which allowed the translocated proteins to be eluted from the organelle membranes. When analysing the eluates, annexin I appears in association with azurophil as well as with specific granules and with secretory vesicles/plasma membrane (Fig. 1). However, also an annexin I-related protein of 38 kDa binds to specific granules and secretory vesicles/plasma membrane in a calcium-dependent manner, whereas this protein does not bind to azurophil granules. As the annexin I of higher molecular mass was the only one present in cytosol, this indicates that the 38 kDa protein, identified by antibodies directed against annexin I, is produced in connection with the translocation process of this protein.

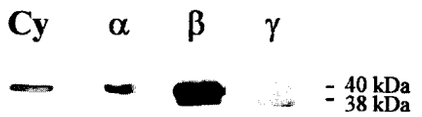

Fig. 1. Identification of annexin I in neutrophil cytosol and translocation of annexin I to neutrophil organelles. Neutrophil cytosol was isolated without EGTA and immunoblotted using monoclonal annexin I antibodies (Cy). Annexin I was translocated to isolated neutrophil organelles (α = azurophil granules, β = specific granules, γ = secretory vesicles/plasma membrane) by incubating them with cytosol in the presence of 2 mM $CaCl_2$. The organelles were collected and washed, then resuspended in 5 mM EGTA to elute calcium-dependent proteins bound to the organelle membranes. Organelles were pelleted and the supernatants assayed by immunoblotting using monoclonal annexin I antibodies.

3.2. Cleavage of annexin I is specifically dependent on calcium

In order to study the formation of the 38 kDa annexin I, cytosol and organelles were allowed to interact simply by mixing them and the appearance of 38 kDa annexin I was detected by immunoblotting. In the presence of calcium, the 38 kDa annexin I appeared together with full size annexin I (40 kDa) (Fig. 2). Since the cytosol was the only source of annexin I in the reaction mixtures, as the organelle preparations did not contain any annexin I (not shown here), we concluded that the 38 kDa molecule was a cleavage product of full size annexin I. Small amounts of the cleaved protein was produced also in the absence of organelles (Fig. 2). The presence of specific granules and secretory vesicles/plasma membrane facilitated the cleavage, whereas the presence of azurophil granules did not. The cleavage was dependent on calcium, which was shown by the fact that if calcium was removed (Fig. 2) or

organelle	-	α	β	γ	α	β	γ
cytosol	+	+	+	+	+	+	+
calcium	+	+	+	+	-	-	-
EGTA	-	-	-	-	+	+	+

Fig. 2. Calcium- and organelle-mediated cleavage of annexin I. Immunoblot detecting annexin I in samples of cytosol which were incubated without (−) or together with isolated azurophil granules (α), specific granules (β), or secretory vesicles/plasma membrane (γ). Samples were incubated for 10 min in the presence of 2 mM $CaCl_2$ or 2 mM EGTA.

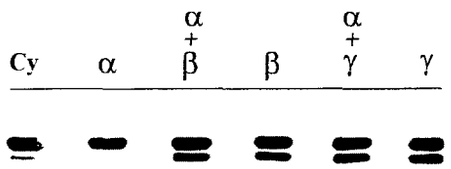

Fig. 3. Are there degrading enzymes in azurophil granules that digest cleaved annexin I? Cytosol was mixed with organelles (α = azurophil granules, β = specific granules, γ = secretory vesicles/plasma membrane) in the combinations indicated. Mixtures were incubated at 37°C in the presence of 2 mM calcium for 60 minutes. The cleaving of annexin I was stopped by addition of EGTA and the samples immunoblotted using monoclonal annexin I antibodies. Cytosol incubated under the same conditions but with organelle preparation omitted was used as a control (Cy).

exchanged for zinc (not shown), then annexin I was not cleaved even though the organelles were present. In fact, the annexin I molecule was very stable. It was still intact after storage of the cytosol for 4 days in 37°C, provided that no calcium was present, and not even when the serine protease inhibitors PMSF and Pefabloc were omitted in the cytosol preparation (not shown). Cleavage of annexin I took place in the presence of protease inhibitors (PMSF, Pefabloc), while the integrity of other annexins was unaffected (tested with antibodies against annexin II, IV and VI). We therefore conclude that cleavage of annexin I requires calcium and that specific granules and secretory vesicles, but not azurophil granules, contain a protease which catalyses specific cleavage of annexin I.

3.3. The inability of azurophil granules to mediate cleavage of annexin I

We hypothesized that the observed inability of azurophil granules to mediate cleavage of annexin I and bind the fragment was artefactual, in that the 38 kDa annexin I is sensitive and presumably becomes degraded by proteases contained in azurophil granules. This possibility was ruled out as the addition of azurophil granules to reaction mixtures containing specific granules or secretory vesicles/plasma membranes did not affect the amount of 38 kDa fragment formed (Fig. 3).

3.4. Cleavage of annexin I by specific granules and secretory vesicles / plasma membrane

The existence of a factor promoting cleavage of annexin I on organelles of the β and γ fraction, was further demonstrated by the increased rate of cleavage of annexin I as the amount of organelles increased (Fig. 4). While as much as a 1000-fold increase in the amount of azurophil granules (compare samples 1 and 4 in Fig. 4A and Fig. 4B) in the reaction mixture did not affect the cleavage of annexin I, stepwise 10-fold increases of specific granules and secretory vesicles/plasma membrane resulted in increased amounts of cleaved 38 kDa protein. Secretory vesicles/plasma membrane contained a higher cleaving capacity than specific granules, in that approximately half of the annexin I was degraded after only 10 min in the presence of a 10-fold excess of organelles over cytosol (sample 4, Fig. 4A).

Kinetical studies also implied the existence of a protease being present in β and γ fractions. While similar kinetics were recorded in samples with azurophil granules as with calcium alone (Fig. 5A), samples with β or γ fractions revealed an increased cleavage of annexin I into the 38 kDa form. Approximately half of the annexin I was cleaved after 75 min at 37°C. Cleavage was somewhat slower with specific granules than in the case of secretory vesicles/plasma membrane.

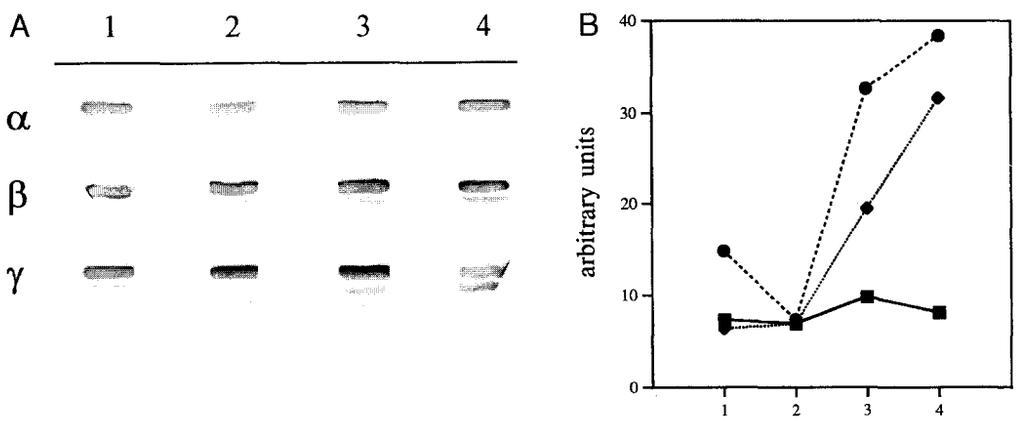

Fig. 4. Difference between the neutrophil organelles in their ability to cleave annexin I. A constant amount of cytosol (2.4×10^6 cell equivalents) was mixed with increasing amounts of azurophil granules (α), specific granules (β), or secretory vesicles/plasma membrane (γ). The amount of organelles added were 2.4×10^4 (1), 2.4×10^5 (2), 2.4×10^6 (3) and 2.4×10^7 (4) cell equivalents. The mixtures were incubated in the presence of 2 mM $CaCl_2$ for 10 min at 37°C. The samples were immunoblotted with monoclonal annexin I antibodies (A), and the immunoblot was quantified by densitometry. The graph (B) shows the amount (ordinate; arbitrary units) of cleaved fragment in each sample; (■) azurophil granules, (♦) specific granules, (●) secretory vesicles/plasma membrane. Abscissa: amount of organelles added to samples 1–4 as above.

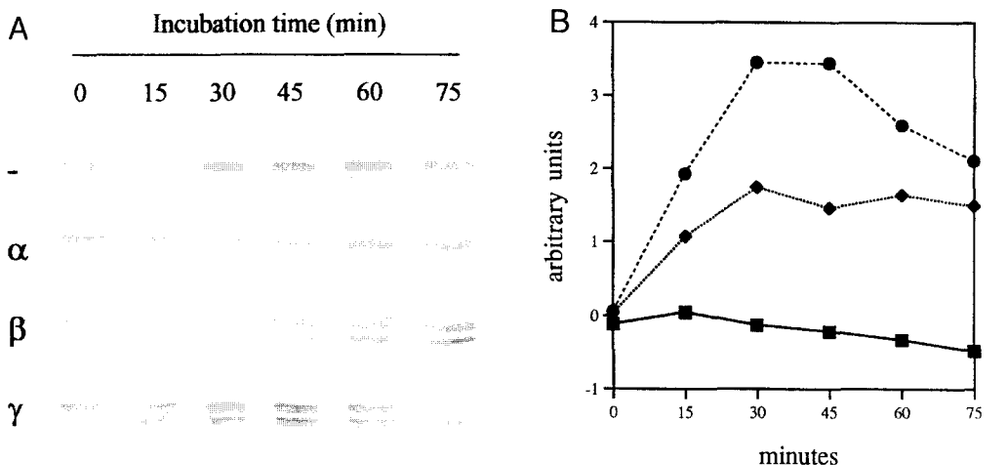

Fig. 5. Kinetics of the degradation of annexin I. Cytosol was incubated at 37°C in the presence of 2 mM $CaCl_2$ either in the absence (−) or presence of azurophil granules (α), specific granules (β) or secretory vesicles/plasma membrane (γ). Aliquots were removed from the samples at time $T = 0$, and then every 15 min. The aliquots were mixed immediately with Laemmli sample buffer and immunoblotted with monoclonal annexin I antibodies (A). The amount of cleaved fragment was quantified by densitometry (B). The values of samples without organelles (−) served as background and were subtracted from each of the corresponding values in the samples containing (■) azurophil granules, (◆) specific granules, (●) secretory vesicles/plasma membrane. Ordinate: amount of cleaved fragment (arbitrary units); abscissa: incubation time (minutes).

3.5. Sequential interaction with organelles

The translocation experiment (Fig. 1) showed that the 38 kDa fragment was not recovered using azurophil granules; an observation that could be related to the inability of these granules to mediate cleavage of annexin I. That experiment did however not allow us to conclude whether this was due only to this inability, or also to an inability of the 38 kDa protein to bind to the azurophil granule membrane. To investigate the latter possibility, we designed a sequential translocation procedure. Cytosol was incubated with an aliquot of organelles, whereby part of the annexin I molecules associated with the organelle membrane. Cytosolic proteins remaining in the supernatant were allowed to bind to a second aliquot of organelles. In this way, the same annexin source, although successively depleted of annexin I, was used for interaction with up to four different portions of organelles.

The use of secretory vesicles/plasma membrane as the first granule encounter, produced a remaining cytosol, which contained the 38 kDa cleavage product (Fig. 6, remaining cytosol II). Using this source of both full size and cleaved annexin I, we were able to show that azurophil granules did not bind the 38 kDa cleavage product even though it was present and available for binding (Fig. 6, sample 2). We therefore conclude that the molecular background to the fact that the 38 kDa annexin I fragment is missing after translocation to azurophil granules is not only due to the inability of these granules to facilitate cleavage, but also to a binding inability of the azurophil granules.

The sequential translocation procedure was also used to clarify further, that the binding of cleaved annexin I seen with specific granules and secretory vesicles/plasma

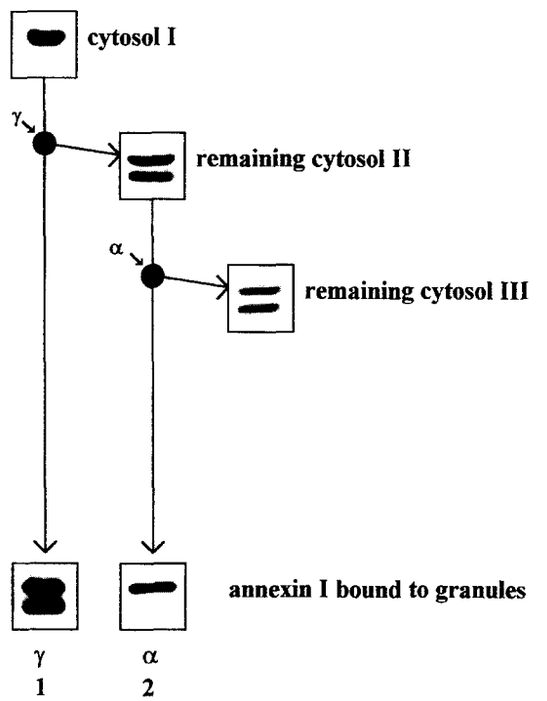

Fig. 6. Inability of azurophil granules to bind 38 kDa annexin I. Isolated cytosol (I) was mixed and incubated with secretory vesicles/plasma membrane (γ) in the presence of 2 mM $CaCl_2$. The mixtures were centrifuged and the supernatant ('remaining cytosol', II) was mixed and incubated with a portion of azurophil granules (α). Centrifugation separated the mixture into 'remaining cytosol' (III) and organelles. The organelles collected (γ, α) were washed and resuspended in 5 mM EGTA to extract proteins calcium-dependently associated with the organelle membranes. After collecting the organelles, the resulting supernatants (1, 2), as well as the cytosols (I, II, III), were immunoblotted using monoclonal annexin I antibodies. Samples designated with Roman numerals show soluble annexin I contained in the cytosol. Samples 1 and 2 show annexin I bound to granules as indicated. ● symbolizes that the cytosol/granule mixtures were incubated and centrifuged.

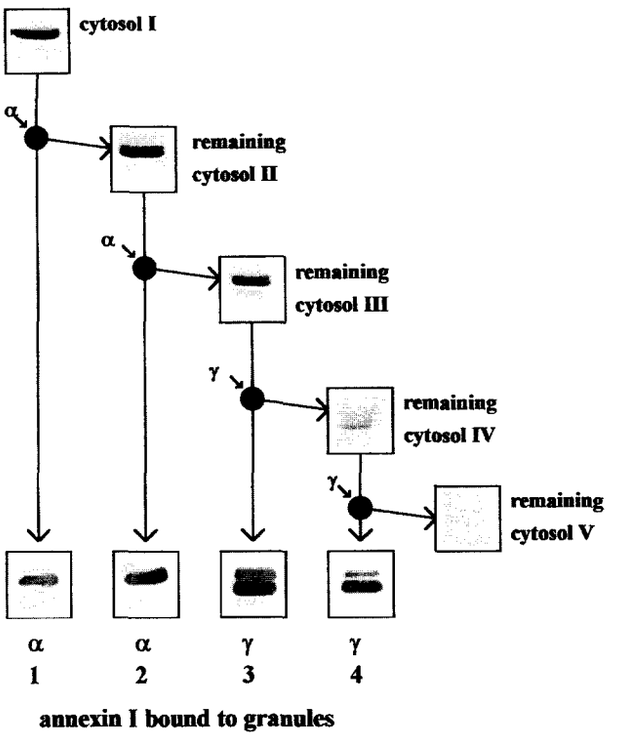

Fig. 7. Binding of cleaved annexin I to secretory vesicles/plasma membrane. The sequential translocation was performed in the same manner as described in figure 6, but the cytosol (I) encountered the organelles in a different order: the first and second portions were azurophil granules (α, 1 and 2), then two portions of secretory vesicles/plasma membrane (γ, 3 and 4). Samples designated with Roman numerals show soluble annexin I contained in the cytosols. Samples 1–4 show annexin I bound to granules as indicated. ● symbolizes that the cytosol/granule mixtures were incubated and centrifuged.

membranes is a true binding, and not only degradation of full-size annexin I occurring during the laborative processing of samples. This was done by first depleting annexin I from the cytosol, by incubation with azurophil granules and then with secretory vesicles/plasma membrane (Fig. 7), until most of the annexin I of the depleted cytosol existed in the truncated form (Fig. 7, remaining cytosol IV). Upon subsequent translocation to secretory vesicles/plasma membrane, it could then be shown that the distribution of full size and cleaved annexin I, respectively, was also reflected when bound to the organelles (Fig. 7, sample 4), in that the truncated form was the predominant one. In fact, all the content of annexin I in the remaining cytosol was able to bind the organelles, as no annexin I was detected in the remaining cytosol (Fig. 7, remaining cytosol V). Similar results were obtained using the β instead of the γ fraction.

4. Discussion

Proteins belonging to the annexin family are among the leading candidates in the search for proteins that promote and regulate membrane fusion events in phagocytic cells. One of these proteins, annexin I, has been shown to promote granule aggregation and modulate fusion in human neutrophils [8]. In this study, we demonstrate that neutrophil cytosolic annexin I is specifically cleaved by a calcium dependent mechanism and that this cleavage is facilitated in the presence of neutrophil specific granules and secretory vesicles/plasma membrane. Our studies also show differences in granule-binding characteristics between the full size and cleaved annexin I, respectively, in that the full-size protein binds to each one type of the organelles isolated, whereas the truncated form binds to the secretory vesicles/plasma membrane and the specific granules but not to the azurophil granules.

The precise mechanism whereby annexin molecules bind to phospholipid vesicles and to natural biological membranes is as yet not fully elucidated. It has been suggested (using annexin V as a model) that annexins, upon binding calcium, form a planar molecule [10], in which calcium acts, on one side of the molecule, to neutralize the negative charges of the acid phospholipids in a membrane, so that the molecule can bind [20]. Whether also other membrane structures are involved in binding is not known. The truncated form of annexin I discriminate between the different neutrophil membranes by binding to specific granules and secretory vesicles/plasma membrane, but rejecting the membrane of azurophil granules. Since the experiments presented rule out the possibility that fragmented annexin I undergoes decomposition in the presence of azurophil granules, it lies near at hand to suggest that the full-size annexin I and the truncated form of the molecule bind to different structures/molecules in the membranes. A possible explanation could be that truncation of the molecule induces conformational changes in the calcium-saturated molecule, that renders it unable to bind directly to phospholipids. Binding of truncated annexin I to membranes could then possibly be aided by a co-factor, present on the specific granules and on secretory vesicles/plasma membrane, which would not be needed for binding of full-size annexin I. The finding that treatment of neutrophil plasma membrane with trypsin inhibited annexin I mediated fusion of neutrophil plasma membranes with liposomes [21] corroborates this suggestion. The fact that neutrophil organelles/membranes differ in phospholipid composition [22–24] may, however, also influence binding characteristics of full-size annexin I and its truncated form. Furthermore, it cannot be excluded that the inability of truncated annexin I to bind azurophil granules may also be an effect of an alteration in calcium sensitivity. Such a phenomenon has been described for truncated annexin I in other biological systems [14,15]. However, in those studies the truncated annexin I was shown to require less calcium for binding.

Cleavage of annexin I has been observed in many other cell types and it has been suggested to be of regulating importance [12,14,25]. Endogenous cathepsin D can medi-

ate cleavage of annexin I [14,15]. Even though cathepsin D is present in the neutrophil, this protease is not likely to be responsible for the cleavage of annexin I described. This conclusion is based on the facts that neutrophil cathepsin D is localized to the azurophil granules (lacking cleavage capacity), and because cathepsin D mediates cleavage of annexin I in the absence of calcium. The cleavage of annexin I in neutrophils was strictly dependent on calcium. The calcium-dependent enzyme calpain can also cleave annexin I [14,15]. Since neutrophils contain calpain, this protease might well be a candidate for the limited cleavage of annexin I observed in the membrane-free cytosol.

Even though this study implies that an annexin I-cleaving factor is present on specific granules and secretory vesicle/plasma membrane, it cannot be excluded that the cleaving factor is cytosolic and that it is either able to translocate to the granule membranes or exert its action at membranes, having a higher affinity for bound than for soluble annexin I. If this is the case, the cleaving factor for some reason must have been unable to exert cleavage at azurophil granules. Future studies will have to establish the true localisation of the cleaving factor, as well as its identity, and at present, we can only conclude that the membrane structure is needed for the facilitated cleavage of annexin I. No cleavage occurred when a detergent was included in the reaction mixture, and this also eliminates the possibility that the cleaving factor originates from the granule matrix.

Taken together, we presently favour the explanation that the cleavage factor is membrane associated. Such an annexin I cleaving protease has been shown to be present in the membranes of A431 cells [25]. Cleavage of annexin I in these cells was reported to be totally dependent on calcium, and it could not be prevented by a number of different inhibitors of well-known calcium-dependent proteases. Phosphorylated annexin I was cleaved by the membrane protease in such a way that the truncated molecule retained the phosphate groups [25], indicating that annexin I was cleaved so that at least one of the four phosphorylation sites was left on the truncated molecule. Thus, the site of cleavage was N-terminally of the 27th amino acid. It remains to be established in our studies, whether the truncated annexin I also bears one or several of the phosphorylation sites.

While the cleavage of annexin I has been described in other cellular systems, the data presented, that the neutrophil granules interact in different ways with annexin I, provides support for the idea that this protein may play a role in differential exocytosis in the neutrophil. In preliminary studies of neutrophils activated by ionomycin, PMA or opsonized yeast, the truncated form of annexin I could not be detected in immunoblots of whole cells. This does neither prove, nor falsify, that the phenomena occur also in situ in the neutrophils since the results can be explained by the possibility that (i) annexin I is further cleaved after the first truncation by a mechanism not occurring in vitro, (ii) cleaved annexin I may be secreted to the extracellular space, (iii) presumptive cleavage in situ may not be detectable due to a low sensitivity of the method used for identification of the cleaved product. Should the in vitro findings reported in this study prove relevant also in vivo in the future, then the data presented provide further support for the hypothesis that annexin I takes part in the exocytic machinery of the neutrophil. Furthermore, annexin I binding to neutrophil organelles may be a good model to disclose the molecular background to the ability of cytosolic proteins to bind selectively to different subcellular organelles.

Acknowledgements

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References

[1] Borregaard, N., Lollike, K., Kjeldsen, L., Sengeløv, H., Bastholm, L., Nielsen, M.H. and Bainton, D.F. (1993) Eur. J. Haematol. 51, 187–198
[2] Raynal, P. and Pollard, H.B. (1994) Biochim. Biophys. Acta 1197, 63–93
[3] Meers, P., Ernst, J.D., Düzgünes, N., Hong, K.L., Fedor, J., Goldstein, I.M. and Papahadjopoulos, D. (1987) J. Biol. Chem. 262, 7850–7858
[4] Sjölin, C., Stendahl, O. and Dahlgren, C. (1994) Biochem. J. 300, 325–330
[5] Ernst, J.D. (1991) J. Immunol. 146, 3110–3114
[6] Homburg, C.H.E., DeHaas, M., Von dem Borne, A.E.G.K., Verhoeven, A.J., Reutelingsperger, C.P.M. and Roos, D. (1995) Blood 85, 532–540
[7] Glenney, J.R., Tack, B. and Powell, M.A. (1987) J. Cell Biol. 104, 503–511
[8] Francis, J.W., Balazovich, K.J., Smolen, J.E., Margolis, D.I. and Boxer, L.A. (1992) J. Clin. Invest. 90, 537–544
[9] Meers, P., Mealy, T. and Tauber, A.I. (1993) Biochim. Biophys. Acta 1147, 177–184
[10] Creutz, C.E. (1992) Science 258, 924–931
[11] Sautebin, L., Carnuccio, R., Ialenti, A. and Di Rosa, M. (1992) Pharmacol. Res. 25, 1–12
[12] Christmas, P., Callaway, J., Fallon, J., Jones, J. and Haigler, H.T. (1991) J. Biol. Chem. 266, 2499–2507
[13] Wang, W. and Creutz, C.E. (1992) Biochemistry 31, 9934–9939
[14] Ando, Y., Imamura, S., Hong, Y.-M., Owada, M.K., Kakunaga, T. and Kannagi, R. (1989) J. Biol. Chem. 264, 6948–6955
[15] Wang, W. and Creutz, C.E. (1994) Biochemistry 33, 275– 282
[16] Bøyum, A. (1968) Scand. J. Clin. Lab. Invest. 97, 77–89
[17] Borregaard, N., Heiple, J.M., Simons, E.R. and Clark, R.A. (1983) J. Cell Biol. 97, 52–61
[18] Laemmli, U.K. (1970) Nature 227, 680–685

[19] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354
[20] Meers, P., Mealy, T., Pavlotsky, N. and Tauber, A.I. (1992) Biochemistry 31, 6372–6382
[21] Oshry, L., Meers, P., Mealy, T. and Tauber, A.I. (1991) Biochim. Biophys. Acta 1066, 239–244
[22] Bjerrum, O.W., Nielsen, H. and Borregaard, N. (1989) Scand. J. Clin. Lab. Invest. 49, 613–622
[23] MacDonald, J.I.S. and Sprecher, H. (1989) J. Biol. Chem. 264, 17718–17726
[24] Wright, D.G., Meierovics, A.I., Richards, R.L. and Alving, C.R. (1981) Fed. Proc. 40, 375
[25] Chuah, S.Y. and Pallen, C.J. (1989) J. Biol. Chem. 264, 21160–21166