Annexin I-Induced Aggregation of Gelatinase Granules in Human Neutrophils

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Ability of neutrophil cytosol to induce aggregation of gelatinase granules from human neutrophils is studied. It is found that cytosol induces Ca^{2+}-dependent aggregation of granules. The aggregation-inducing capacity of cytosol considerably decreases in the presence of monoclonal antibodies to annexin I, a transmitter of Ca^{2+}-dependent aggregation of gelatinase granules probably involved in their secretion.

Key Words: *neutrophil; annexin; gelatinase granules; degranulation*

Human neutrophils (polymorphonuclear leukocytes) contain 4 types of granules: azurophilic, specific, and gelatinase granules (GG), and secretory vesicles, which are characterized by different mobilization capacity in response to increased cell Ca^{2+} concentration [4,6]. Rapid mobilization of GG during phagocytosis is important for the development of early neutrophil response to stimulation, in particular, for adhesion to endothelium, transendothelial migration, and migration to the inflammatory focus [10]. Molecular mechanisms of regulation of GG exocytosis are unknown. Previous experiments demonstrated the involvement of annexins in secretion of neutrophil granules [5, 7,8,11]. Annexins are a multigene family of Ca^{2+}-dependent phospholipid-binding proteins. In the presence of micromolar Ca^{2+} concentrations these proteins bind to phospholipid bilayers, while in the presence of higher Ca^{2+} concentration they induce aggregation of natural and model membranes [2]. It was found that annexin I induced aggregation of specific neutrophil granules and fusion of specific granules with plasma membrane vesicles [8].

In the present study we investigated the effect of annexin I, a component of neutrophil cytosol, on Ca^{2+}-dependent aggregation of GG.

MATERIALS AND METHODS

The rate of aggregation was evaluated in the presence of monoclonal antibodies to the N-terminal region of annexin I (Zymed). For isolation of cytosol, human peripheral blood neutrophils (10^8 cells) were suspended in 2 ml 50 mM Tris-HCl (pH 8) containing 100 mM NaCl, 2 mM EGTA, and 2 mM PMSF, homogenized in a Potter homogenizer, and centrifuged at 125,000*g* for 90 min. The supernatant was dialyzed against 50 mM Tris-HCl (pH 7.2) containing 100 mM NaCl (buffer A). Fraction of GG was isolated by subcellular fractionation of neutrophils and identified by the presence of gelatinase [9].

Aggregation of granules was assessed by optical density increment (540 nm) during 5 min after addition of an inductor [3]. The suspension of GG was adjusted with buffer A containing 250 mM sucrose to optical density of 0.2 units (540 nm), and 1.5 ml suspension was added to 0.5 ml 8 mM $CaCl_2$ or 8 mM EGTA. Aggregation was initiated by adding the cytosol (200 μg protein per 100 μl). The concentration of cytosol protein was chosen empirically. The data (mean of 3 independent experiments) were presented as a dependence of DA_{540} (difference between current and initial A_{540}) on the time of incubation.

RESULTS

Human neutrophils were subjected to subcellular fractionation under conditions allowing to isolate individual populations of cytoplasmic granules. The distribution of enzyme activity observed in our experiments corresponded to that observed under similar fractiona-

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tion conditions [9]. Fraction 4 enriched with GG was used in further experiments.

Cytosol (200 μg protein) added to suspended granules induced aggregation of GG in the presence of 2 mM $CaCl_2$ in the incubation medium (Fig. 2). In the absence of free Ca^{2+} (2 mM EGTA) cytosol had no effect on optical density of the suspension. It was shown that natural and model biological membranes aggregate in the presence of 1 μM or higher concentration of Ca^{2+} [1]. Therefore we studied aggregation in the presence of 2 mM $CaCl_2$. Ca^{2+} alone (without cytosol) had no effect on optical density of the incubation mixture (Fig. 2). Hence, Ca^{2+}-dependent aggregation of GG is induced by cytosolic factors.

Monoclonal antibodies considerably inhibited aggregation (Fig. 2). Hence, cytosol induces Ca^{2+}-dependent aggregation of GG via annexin I. It should be noted that optical density of the suspension slightly increased during the first minute of incubation. We assumed that the concentration of antibodies is insufficient to block all annexin I molecules. However, the aggregation curves remained unchanged after the concentration of antibodies was 2-fold increased. Previous studies demonstrated translocation of annexins I, II, III, IV, VI, and XI molecules from the cytosol to granule membranes via a Ca^{2+}-dependent mechanism [5,11]. It can be hypothesized that apart from annexin I, other proteins of this family as well as non-annexin cytoplasmic factors can contribute to GG aggregation.

Thus, we demonstrated induction of Ca^{2+}-dependent aggregation of GG from human neutrophils by annexin I, which suggested possible involvement of this protein in mechanism of membrane adhesion/fusion during Ca^{2+}-regulated exocytosis of GG.

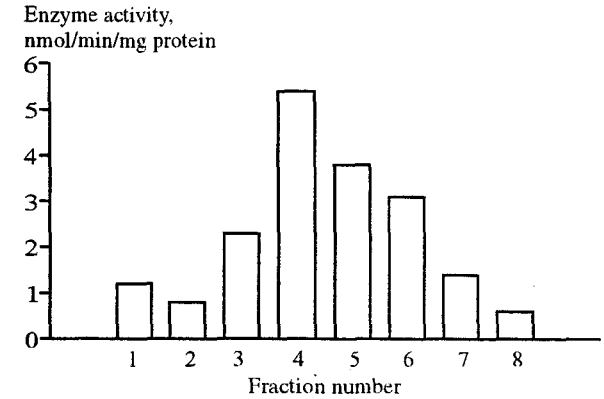

Fig. 1. Gelatinase activity in subcellular neutrophil fractions.

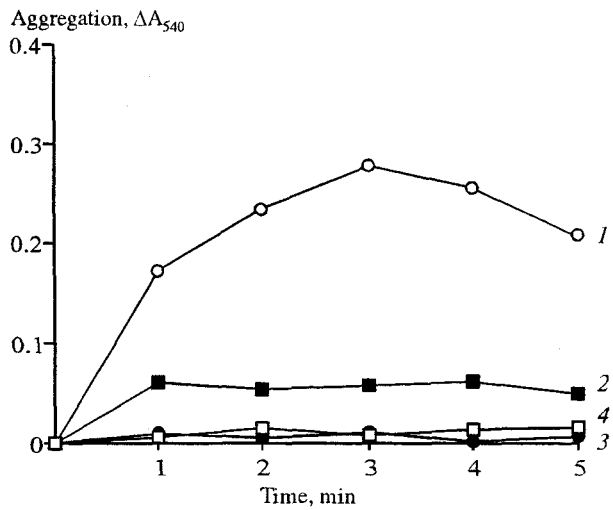

Fig. 2. Rate and degree of gelatinase granule aggregation in the presence of cytosol and 2 mM $CaCl_2$ (*1*); cytosol, 2 mM $CaCl_2$ and monoclonal antibodies to annexin I (1 μg/ml, *2*); 2 mM $CaCl_2$ (*3*); cytosol and 2 mM EGTA (*4*).

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