Intracellular Localization of VAMP-1 Protein in Human Neutrophils

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We studied the intracellular localization of vesicle-associated membrane protein VAMP-1 in human neutrophils. VAMP-1 was associated with membranes of gelatinase and specific secretory granules rapidly mobilized during exocytosis. VAMP-1 probably acts as a component of the SNARE complex during exocytosis of gelatinase and specific granules in human neutrophils.

Key Words: *neutrophil; secretory granules; exocytosis; SNARE; VAMP-1*

Realization of the protective and inflammatory functions of human neutrophils (polymorphonuclear leukocytes) is related to mobilization of intracellular granules. Exocytosis of granules is necessary for adhesion and transendothelial migration of cells, formation of reactive oxygen species, and secretion of lytic enzymes. The molecular mechanisms underlying exocytosis in human neutrophils are poorly understood. Recently, this process was viewed in terms of the fusion/adhesion model initially used to study exocytosis of synaptic vesicle (SNARE hypothesis). This model is based on the interaction between vesicular membrane protein VAMP (vesicle-associated membrane protein) and plasma membrane proteins syntaxin and SNAP-25 (25-kDa protein associated with synaptosomes), formation of SNARE complexes, binding to cytosolic proteins, and membrane fusion [3,4].

Human neutrophils contain various isoforms of key proteins of the SNARE complex. Therefore, in these cells exocytosis is realized by the molecular mechanism described by the SNARE hypothesis [2, 6,9]. Human neutrophilic VAMP-2 protein belongs to the family of VAMP proteins and is probably involved in the secretion of gelatinase and specific granules [2]. Here we performed immune detection of VAMP-1 protein in human neutrophils and studied its intracellular localization.

MATERIALS AND METHODS

Neutrophils were isolated from human peripheral blood by erythrocyte sedimentation with dextran, centrifugation in a Ficoll-Hypaque density gradient, and hypotonic lysis of erythrocytes [8]. Neutrophils were suspended in 50 mM Tris-HCl buffer (pH 7.5) containing 2 mM phenylmethylsulfonyl fluoride and disintegrated by homogenization in a Potter homogenizer. The homogenate was centrifuged at 2000*g* for 10 min, and the postnuclear supernatant was collected. The cytosolic and membrane fractions were isolated by centrifugation at 125,000*g* for 90 min. The extract was centrifuged in a 15-40% sucrose linear density gradient [8]. Fractions were identified by the content of marker proteins: the cytosol (fraction 1), plasma membrane (fractions 2 and 3), and gelatinase (fraction 4), specific (fractions 5 and 6), and azurophilic granules (fractions 7 and 8) were isolated [8]. Membranes were precipitated by centrifugation of subcellular fractions at 125,000*g* for 90 min. Extract, cytosol, and membrane samples and membranes of subcellular fractions (30 μg protein) were subjected to electrophoresis in 15% PAAG [5] and immunoblotting with monospecific polyclonal antibodies 18.1 against VAMP-1 [10] gifted by Dr. H. Blazi (Barcelona University,

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Spain). Polyclonal antibodies against recombinant VAMP-1 are highly specific for VAMP-1 in neuronal and neuroendocrine cells [11]. Unpurified extract from rat brain (3 μg protein) containing at least 3 isoforms of VAMP proteins, including VAMP-1, served as the positive control [7,11].

RESULTS

Only 1 band corresponding to the protein with a molecular weight of 18 kDa was detected in the neutrophil extract (Fig. 1). The electrophoretic mobility of this VAMP-1-immunoreactive protein from human neutrophils was similar to that of VAMP-1 from rat brain. Studies of the cytosolic and membrane neutrophil fractions revealed the presence of this protein in cell membranes, but not in the cytosol. Membrane topology of VAMP-1 in human neutrophils is consistent with published data. This protein is associated with membranes of secretory vesicles in neuronal and neuroendocrine cell with regulated secretion [11].

Immunoblotting of subcellular neutrophil fractions revealed VAMP-1 in gelatinase and specific granules (Fig. 2, *4-6*). Human neutrophils contain 4 types of granules (azurophilic, specific, and gelatinase granules and secretory vesicles) displaying various exocytotic reactions to an increase in intracellular Ca^{2+} concentration [1]. VAMP-1 is present in rapidly mobilized granules, but not in slow mobilized azurophilic granules. Intracellular localization of VAMP-1 indicates its involvement in regulated exocytosis in human neutrophils. The presence of 2 VAMP isoforms in human neutrophils (VAMP-1 and VAMP-2) [2] suggests that they are involved in *in vivo* exocytosis of various granules and/or act as the components of the SNARE complexes in cells during initiation of exocytosis.

Thus, human neutrophils contain VAMP-1 protein associated with membranes of gelatinase and specific granules.

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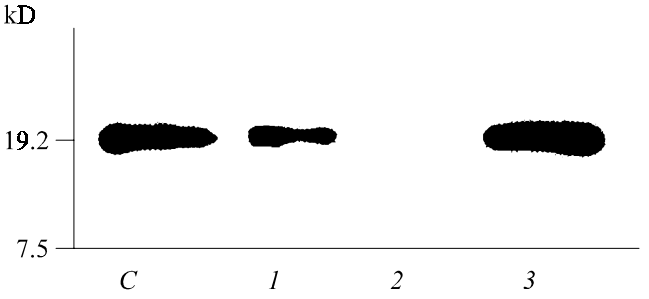

Fig. 1. PAAG electrophoresis and immunoblotting of postnuclear (*1*), cytosolic (*2*), and membrane (*3*) fractions of human neutrophils. Control (*C*): rat brain extract (3 μg protein).

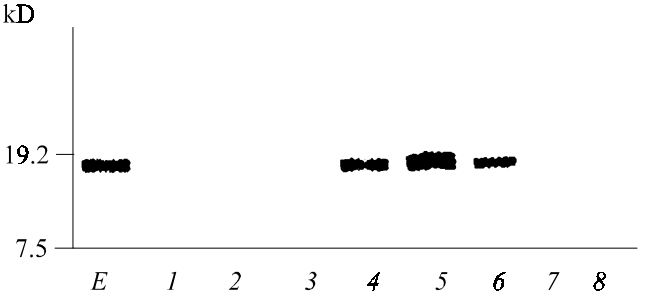

Fig. 2. PAAG electrophoresis and immunoblotting of postnuclear (*E*, 15 μg) and subcellular fractions (*1-8*) of human neutrophils.

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