Interaction of rat liver lysosomes with basic polypeptides

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Abstract In order to gain knowledge on the interaction of lysosomes with proteins, we have assessed the equilibrium densities of the lysosomal membrane and matrix markers after in vitro incubation of rat liver lysosomes with various polypeptides. The addition of basic polypeptides, polylysine or protamine, to the suspension of lysosomes brought about a profound alteration of lysosomal membrane, causing extensive leakage of lysosomal matrix enzymes. Electron microscopic observation revealed a remarkable aggregation of lysosomes by the basic polypeptides. Polyglutamic acid, an acidic polypeptide, did not produce such effect. ATP was found to stabilize lysosomes during incubation, particularly with basic polypeptides.

Key words: Lysosome; Stability; ATP; Interaction with polypeptides

1. Introduction

Lysosomes are known to play an important role in intracellular protein degradation [1,2]. Recent studies in vivo [2–5] and in vitro [5,6] indicate that intracellular proteolysis may involve selective sequestration of certain proteins into lysosomes.

In order to gain some insight into the mode of interaction of lysosomes with proteins, we have investigated the interaction of isolated rat liver lysosomes with several polypeptides. We have found that lysosomes specifically bind basic polypeptides such as polylysine and protamine, and that ATP serves to stabilize the lysosomes during interaction with these basic polypeptides.

2. Materials and methods

2.1. Materials

Percoll and density marker beads were purchased from Pharmacia LKB (Sweden). Creatine phosphate and creatine phosphokinase were from Boehringer-Mannheim (Germany). Poly-L-lysine (MW > 8000) and poly-L-glutamic acid (MW > 8000) were from Peptide Institute (Osaka, Japan). FITC-labeled poly-L-lysine (0.0044 mol FITC/mol lysine monomer), protamine, 4-methylumbelliferyl-β-D-galactoside and 4-methylumbelliferyl-β-D-glucoside were from Sigma (St. Louis, USA).

2.2. Isolation of lysosomes from rat liver

Highly putified lysosomes were isolated from the liver of male Wistar rats by the method developed in our laboratory [7]. In some experiments, isolated lysosomes were subjected to several cycles of freezing and thawing, and used as disrupted lysosomes.

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2.3. Isolation of lysosomal membranes

Lysosomal membranes were prepared from the isolated lysosomes after hypoosmotic shock followed by treatment with 1 M NaCl and centrifugation at 105,000 × *g* for 1 h as described by Yamamoto et al. [8].

2.4. Incubation condition

The freshly isolated lysosomes (200 μg of protein) were incubated with 100 μg of various polypeptides in 0.25 M sucrose containing 20 mM MOPS buffer (pH 7.0), with or without 5 mM Mg^{2+}-ATP and ATP-regenerating system (10 mM creatine phosphate and 30 units/ml of creatine phosphokinase) in a final volume of 150 μl. After incubation at 37°C for 30 min, the incubation mixtures were layered on the top of preformed Percoll gradients in Eppendorf tubes (described below) and centrifuged at 15,000 rpm for 60 min in a Hitachi Himac centrifuge (CR 15D). After the centrifugation, fractions of about 120 μl were collected by piercing the bottom of the tubes under the pressure made by a perista pump and analyzed for the activities of lysosomal marker enzymes. For the preparation of performed Percoll gradients, Eppendorf tubes containing 1.3 ml of 37% Percoll in 0.25 M sucrose (pH 7.0) were centrifuged at 15,000 rpm in a Hitachi Himac centrifuge for 4 h. Density profiles of the Percoll gradients were determined by using density calibration beads in the range of 1.035–1.149.

2.5. Assay of lysosomal marker enzymes

Activities of β-glucosidase (marker enzyme for lysosomal membrane) and β-galactosidase (marker enzyme for lysosomal matrix) were measured by the method of Barrett [9] using fluorogenic methylumbelliferyl substrates.

2.6. Binding of lysosomes and lysosomal membranes to FITC-labeled polylysine

Lysosomes and lysosomal membranes (200 μg of protein each) were incubated with FITC-labeled polylysine (0.1 mg/ml) in the presence of 5 mM Mg^{2+}-ATP and ATP-regenerating system under the same condition as described above at 37°C for 30 min. The centrifugation in the preformed Percoll gradients and collection of the fractions were also performed in the same manner as described above. The fluorescence in each fraction was measured by fluorometry, with excitation at 495 nm and the emission at 515 nm.

2.7. Electron microscopy

Electron microscopic observation of lysosomes was performed as described previously [7].

3. Results

Isolated lysosomes were incubated at 37°C for 30 min in the presence or absence of 5 mM ATP and subjected to isopycnic centrifugation in an iso-osmotic (0.25 M sucrose) preformed Percoll density gradient. After fractionation into 10 fractions, each fraction was assayed for the activities of β-glucosidase (β-Glu) and β-galactosidase (β-Gal) which served as the marker enzymes of lysosomal membrane and lysosomal matrix, respectively. As illustrated in Fig. 1A and E, the major peaks of both

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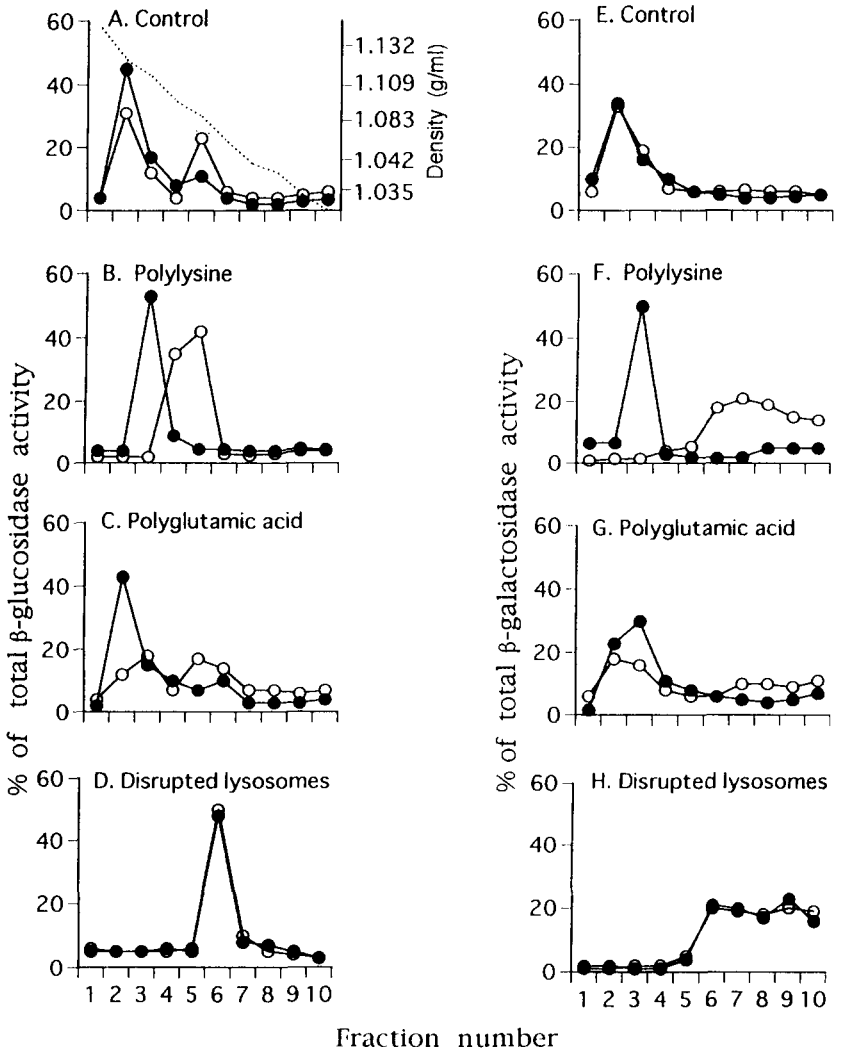

Fig. 1. Change in lysosomal equilibrium density caused by in vitro incubation with various polypeptides in the presence or absence of ATP. The isolated rat liver lysosomes were incubated with various polypeptides as described in section 2 in the presence (●) or absence (○) of ATP and ATP-regenerating system. The incubated samples were fractionated in the preformed Percoll density gradient. The density of each fraction (···), determined by density calibration beads, was very reproducible in all tubes and depicted only in panel A. The experiments shown are representative of three independent experiments.

marker enzymes were banded at the density of 1.12 g/ml which represented the intact lysosomes. However, the lysosomes when incubated in the absence of ATP showed an additional minor peak of β-Glu at 1.08 g/ml (Fig. 1A). This minor peak probably represents partially disrupted lysosomes from which a part of the matrix material has leaked out. It thus appears that the presence of ATP during the incubation protects the lysosomes from destabilization of the membrane.

When polylysine, a basic polypeptide, was added to the incubation mixture in the presence of ATP, the peaks of β-Glu and β-Gal were displaced by one fraction toward a lighter density of 1.10 g/ml (Fig. 1B and F). In contrast, the addition of polylysine in the absence of ATP showed a remarkable effect on the integrity of lysosomes. The membrane marker enzyme (β-Glu) was shifted to 1.08 g/ml and the matrix marker enzyme (β-Gal) was dispersed in the region less than 1.05 g/ml, indicating an extensive leakage of the lysosomal matrix due to a damage of lysosomal membrane. The incubation of lysosomes with protamine, another basic polypeptide, at the same concentration as polylysine, produced a similar damage to the lysosomes as polylysine (data not shown). However, low-molecular compounds like spermine and lysine at 5 mM concentration did not exert any effect on the lysosomal stability (data not shown).

The incubation of lysosomes with polyglutamic acid, an acidic polypeptide, produced a very modest effect on the lysosomal stability; the density profiles of the marker enzymes were similar to those of the controls (Fig. 1C and G). The protective effect of ATP was also evident.

Disruption of lysosomal membranes by freezing and thawing caused density shifts of the membrane marker to 1.05 g/ml and the matrix marker to the region less than 1.05 g/ml (Fig. 1D and H). The presence or absence of ATP during the incubation had no effect on the density profiles.

The electron micrographs of lysosomes after incubation with and without polylysine are shown in Fig. 2. As seen in Fig. 2B, the incubation with polylysine brought about a remarkable aggregation of lysosomes. The incubation was carried out in the presence of ATP. One should therefore note that, although the density shift by polylysine was only modest after incubation in the presence of ATP (Fig. 1B and F), the lysosomes underwent

an extensive morphological change during the incubation with polylysine. Essentially the same result was obtained when lysosomes were incubated with protamine (data not shown).

Finally, we examined if polylysine actually binds to lysosomes during the incubation. For this purpose, we employed FITC-labeled polylysine. Intact lysosomes and lysosomal membrane fraction were incubated with FITC-polylysine in the presence of ATP. As shown in Fig. 3, the peaks of FITC-label were observed at the density of 1.12 g/ml in the case of intact lysosomes and at 1.05 g/ml in the case of lysosomal membrane. When FITC-polylysine alone was centrifuged, in the absence of lysosomal samples, most of the FITC-label was found on the top of the gradient (fractions 9 and 10) (data not shown). We thus conclude that polylysine indeed binds to lysosomal membrane.

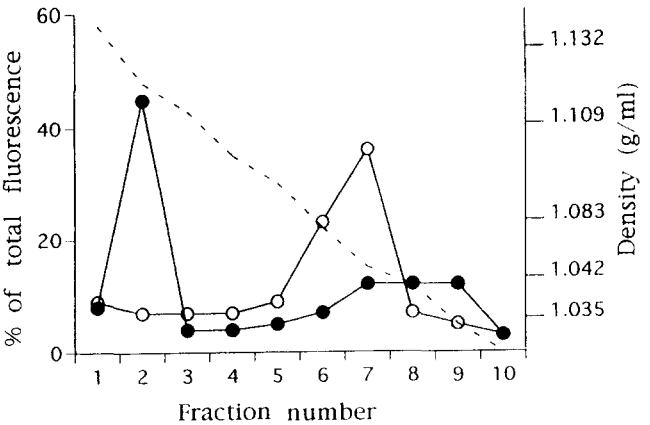

Fig. 3. Binding of FITC-polylysine to intact lysosomes (●) and lysosomal membrane (○). Intact lysosomes or lysosomal membrane were incubated with FITC-polylysine in the presence of ATP and ATP-regenerating system as described in section 2. The incubated samples were fractionated as in Fig. 1 and the fluorescence in each fraction was measured as described in section 2. The density of each fraction (---) was estimated by density calibration beads. The experiments shown are representative of three independent experiments.

4. Discussion

The present study shows that addition of basic polypeptides, polylysine or protamine, to the suspension of lysosomes causes extensive aggregation of lysosomes and leakage of lysosomal matrix. Polyglutamic acid, an acidic polypeptide, does not produce such effect.

The aggregation of a variety of small vesicles made either of natural biological membrane or of phospholipid or phospholipid–protein mixtures by the basic polypeptides, polylysine or protamine, have been described [10]. More recently, aggregation of erythrocyte membrane by polylysine have been reported [11]. Although the mechanism of aggregation is not completely understood, it has been proposed that the aggregation works by binding of positively charged lysine or arginine groups to acidic phospholipid groups (or negatively charged groups on membrane proteins where such are present) in the membrane of the vesicles and thereby binds the vesicles together. The present demonstration of the binding of FITC-polylysine to lysosomal membrane may substantiate this idea.

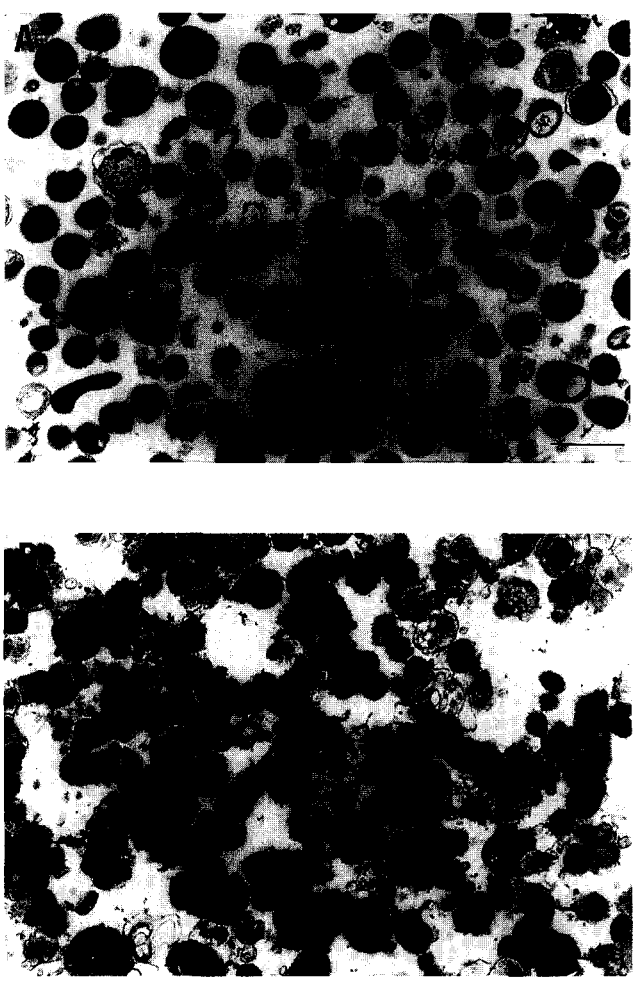

Fig. 2. Electron micrographs of lysosomes, incubated in the absence (A) or presence (B) of polylysine. Bar = 1.0 μm.

If membrane proteins are involved in the lysosomal aggregation, an attractive candidate would be a group of lysosomal membrane glycoproteins called lamp proteins [12]. One difficulty with this possibility, however, is that large parts of these molecules are located in the lumenal side of lysosomes and only small parts are exposed to the cytoplasmic side.

The present study also shows that ATP effectively stabilizes lysosomes during in vitro incubation, particularly when lysosomes are incubated with basic polypeptides such as polylysine. ATP does not seem to act by preventing the association of polylysine with the lysosomal membrane since the polylysine binding was demonstrated in the presence of ATP. In somewhat different context, Mayorga et al. [13] have recently reported that ATP prevents the density shift of lysosomes caused by incubation at low pH but the mechanism of the ATP action has remained unknown.

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