Reactivity of Lysosomes to Inside-out Cell Membrane Vesicles in a Cell-free System

Norio Kawai and Ichiro Ichihara
Department of Anatomy, Aichi Medical University, Nagakute-cho, Aichi 480-11, Japan

Key words: lysosome reactivity/cell membrane/inside-out vesicle/fusion/acellular system

ABSTRACT. To study ultrastructurally the mechanisms of lysosome reactions to cell membrane-derived intracellular membranes we developed a cell free system using small inside-out and rightside-out cell membrane vesicles (IOVs and ROVs) as a target of the reactions. The IOVs were generated from rat erythrocyte ghosts in a low ionic strength alkaline solution in the absence of divalent cations after erythrocytes were reacted with wheat germ agglutinin-coated colloidal gold [WGA (CG)], while ROVs were from ghosts homogenized in a buffer with MgSO4 and bovine serum albumin-coated CG [BSA (CG)]. WGA (CG) bound to the cell surface were rear- ranged on the membrane and distributed irregularly on the inner surface of generated small IOVs. A coat structure derived from the ghost's submembranous coat was almost depleted from their outer surface. By contrast, BSA (CG)-binding to the membranes was negligible in the process of ROV formation. When isolated rat liver lysosomes were incubated with these WGA (CG)-binding small IOVs at 37°C, CG particles were found in several lysosomes under electron microscopy. Some lysosomes adhered to the IOVs, and their limiting membranes were found to collapse and disappear partially at the adhering region, suggesting their fusion. This reaction seems to occur even in cytosol-free solution. By contrast, the lysosomes indicated very low reaction to BSA (CG)-containing ROV, and to WGA (CG) or BSA (CG) alone. Therefore, it is suggested that isolated liver lysosomes react, at least to fuse, in a cytosol-independent fashion, with surface coat-depleted IOVs derived from WGA (CG)-bound and then -rearranged erythrocyte membranes.

The lysosomal system plays an important role in disposition of intracellular substances in cellular heterophagy and autophagy. A variety of cell membrane-bound macromolecular ligands and fluid-phase molecules are endocytosed and mostly degenerated by the lysosome in many types of cells. Early processes in receptor-mediated endocytosis have been reported to be characterized by a series of steps involving rearrangement of receptor-ligand complexes on the cell membrane, their accumulation at coated pits and then coated vesicle formation (10). The ligands in these vesicles seem to be delivered to endosomes by a specific membrane fusion after their vesicle's uncoating (14, 39). Lysosomes have been suggested to interact with membranes of endosomes or some other cytoplasmic components to fuse with or wrap around them (14, 25, 32, 37). Several workers suggested a correlation of cytoskeletal proteins with lysosomal mobility in vivo (15, 21) and their reaction with lysosomes in vitro (22, 23). Recently, some attempts have been made in cell free systems to clarify the mechanisms of endocytic pathway events. In these systems, enzyme-substrate or immunological reactions, or a radio-labeled method were used to indicate fusion between isolated endocytic compartments. Each of them was labeled in vivo by the enzyme and so on before their isolation (1, 5, 7, 11, 24, 40, 41). These systems seem to require provisions of undamaged cytosol and an ATP regenerating system in the fusion. However, molecular events involved in the endocytic pathways have been less well understood except for the coated vesicle's coat (6, 26, 28). It has been obscure which membrane architectures in the processes are related to the lysosome-phagosome interaction. We examined another acellular system without cytosol for analysis of the lysosome reaction to cell membrane-derived intracellular membranes using small vesicles generated from erythrocyte plasma membranes which had been bound by colloidal gold-labeled macromolecular ligands. This paper reports an interaction in the absence of cytosol, at least fusion, between isolated rat liver lys-
somes and inside-out vesicles (IOV) derived from the ligand-bound and then -rearranged cell membranes.

**MATERIALS AND METHODS**

**Reagents.** Wheat germ agglutinin-coated or ferritin-labeled colloidal golds [WGA (CG) or WGA (Fer)] (E-Y Laboratories, San Mateo, CA) were used as macromolecular ligands and markers for cell membranes. Bovine serum albumin (tissue culture grade, Ito Ham CO. Ltd., Tokyo, Japan) -coated colloidal gold (E-Y Laboratories) [BSA (CG)] was prepared basically according to the method of De Roe et al. (8). BSA was treated for 25 min at 60°C before the coating. The diameter of used colloidal golds was 15 ± 3 nm. Rabbit anti-rat erythrocyte IgG was obtained from Inter-Cell Technologies, Inc. (Hopewell, NJ). Leupeptin, pepstatin and chymostatin were purchased from Peptide Institute Inc. (Osaka, Japan), and aprotinin, phenylmethylsulfonyl fluoride, ATP, creatine phosphate and creatine phosphokinase were from Sigma (St. Louis, MO).

**Reaction of WGA (CG), WGA (Fer) and BSA (CG) to erythrocyte membranes.** Freshly drawn blood obtained from male Wistar rats (200–300 g) was anticoagulated with Citral (Yamanouchi Pharmaceutical Co. Ltd., Tokyo, Japan) and washed three times (500 g-sedimentation) with ice cold PBS. Some washed erythrocytes were prefixed with 2.5% glutaraldehyde (GLA) in PBS for 30 min at 4°C and rewashed. These unfixed and prefixed erythrocytes, prepared from 0.1 ml fresh blood (about 10⁶ erythrocytes) respectively, were incubated with WGA (CG), WGA (CG) and 0.5 M N-acetyl-D-glucosamine (GlcNAc), or BSA (CG) in PBS for 30 min at 37°C or 4°C. The concentrations of WGA (CG) and BSA (CG) were 45 μg WGA and 70 μg BSA per ml, respectively. After re-washing, they were fixed with 1% osmium tetroxide in 0.1 M phosphate buffer at pH 7.3 for 30 min at 4°C, dehydrated with graded ethanol, embedded in Epon 812, and then processed for transmission electron microscopy (TEM).

Erythrocyte plasma membranes (RBC ghosts) of the rat were prepared in the 10 mM Tris-HCl buffer at pH 7.4 (buffer 1). Some washed ghosts were similarly GLA-prefixed. These unfixed- and prefixed-ghosts, prepared from 10⁶ cells, were incubated with WGA (CG), WGA (CG) and GlcNAc, or BSA (CG) in PBS or the buffer 1 for 30 min at 4°C or 37°C. Each concentration of the protein-golds and GlcNAc is the same as that in the incubation with RBC. These incubated ghosts were washed with each cold buffer, postosmicated and then processed for TEM as described above.

WGA- and BSA-binding patterns were estimated by counting the total and clustered CG particles on the membrane, respectively. The number of the particles and their percentage of their clustering were calculated for each unit zone (1 μm length) of ultrathin-sectioned (about 50 nm in thickness) membranes. Obliquely sectioned and unclearly identified membrane zones were omitted from the calculation.

**Preparation of WGA (CG)-bound or BSA (CG)-labeled small vesicles [WGA (CG)- or BSA (CG)-vesicles] from RBC ghosts.** RBC ghosts were reacted with WGA (CG) at the WGA concentration of 45 μg/ml in the buffer 1 at 37°C for 30 min. WGA (CG)-treated ghosts were washed with ice cold 10 mM Tris-HCl buffer, pH 8.0, containing 0.1 mM EDTA, incubated in the same solution at 37°C for 1 h because of induction of IOV formation, and then passed five times through a 27 gauge (G) hypodermic needle of a syringe. Following centrifugation of 18,000 g for 30 min, the precipitates were suspended in ice cold PBS with 0.2 mM MgSO₄ and mixed with rat RBC and then with the same buffer containing 0.48 mg/ml of anti-rat RBC antibodies (1:10) at 4°C. After shaking at room temperature for 30 min, they were sedimented at 100 g for 5 min. The same incubation and sedimentation of the supernatant were further repeated several times to remove right-side-out vesicles (ROVs). The resulting supernatant was centrifuged at 2,000 g for 15 min and then at 18,000 g for 30 min. These 18,000 g-precipitates [WGA (CG)-vesicle fraction] were suspended in cold 10 mM HEPES with 0.25 M sucrose, 0.2 mM MgSO₄ and 0.2 M KCl, pH 7.0 (buffer 2).

ROVs were prepared by passages through 27 G needle and micropipettes (100–200 and 10–50 μm in inside diameter) of RBC ghosts in 2.5 mM K⁺-HEPES buffer (pH 7.6) with 0.2 mM MgSO₄ and with BSA (CG) (47 μg BSA/ml) at 4°C, and then by incubation at 37°C for 1 h. They were centrifuged at 4,000 g for 20 min. Following re-centrifugation of the resulting supernatant at 18,000 g for 30 min, the precipitates [BSA (CG)-vesicle fraction] were resuspended in cold buffer 2.

Intact RBC ghosts, and some samples of WGA (CG)- and BSA (CG)-vesicle fractions were prefixed by 0.2% tannic acid-2.5% GLA in 0.1 M sodium cacodylate buffer, pH 7.4, at 4°C overnight, washed, postosmicated and then processed for TEM, as described above.

To estimate the rates of IOVs, ROVs and nonsvesiculated membrane fragments with WGA (CG) or BSA (CG), they were calculated at each area of ultrathin-sectioned samples.

**WGA (Fer)-binding to RBC ghosts, and WGA (CG) and BSA (CG)-vesicles.** Intact RBC ghosts, and WGA (CG)- and BSA (CG)-vesicle fractions (prepared from 10⁶ cells, respectively) were GLA (2.5%)-prefixed, washed and then incubated with WGA (Fer), or WGA (Fer) and 0.3 M GlcNAc in PBS for 30 min at 37°C. The concentration of WGA (Fer) was 0.5 mg protein/ml. After washing, they were postosmicated, dehydrated and then embedded in Epon 812 for TEM. The binding of WGA (Fer) to the outer surface of these membranes was examined in their ultrathin-sectioned samples.

**Preparation of lysosomes.** Lysosomes were isolated from the rat liver according to the method described by Tsuji et al. (33) with the following modifications. All buffers (pH 7.0) included 10 mM HEPES and protease inhibitors; 5 μM leupeptin, 5 μM pepstatin, 5 μg/ml chymostatin, 5 μg/ml aprotinin and 1 mM phenylmethylsulfonyl fluoride. In addition, a glass/Teflon Potter-Elvehjem homogenizer with a very loosely fitting Teflon pestle was used instead of a Waring blender. Small pieces of the liver were carefully homogenized at 4°C in
the buffer using several strokes (by hand) of the homogenizer. After several centrifugations, the prepared lysosomes (lysosome fraction) were washed with cold buffer 2. Some samples of this fraction were then subfractionated by isopycnic centrifugation in metrizamide gradients or Percoll gradients (4, 22).

**Reaction of lysosomes to WGA (CG)-vesicles, BSA (CG)-vesicles, WGA (CG), or BSA (CG).** The lysosome fraction prepared from 0.25 g (wet weight) liver was incubated with WGA (CG)-vesicles, BSA (CG)-vesicles, WGA (CG) or BSA (CG) in buffer 2 with or without an ATP regenerating system [freshly prepared 1 mM ATP, 8 mM creatine phosphate, 30 units/ml creatine phosphokinase and 1.5 mM Mg(OAc)₂] for 40 min at 37°C. Each experimental group included the vesicle fraction originating from 0.3 ml fresh blood, 45 µg WGA/ml in WGA (CG) or 70 µg BSA/ml in BSA (CG). All these reactions were examined in the absence of cytosol. Ten thousand g (20 min)-precipitates of them were washed with cold buffer 2, fixed by 2.5% GLA, postfixed by 1% osmium tetroxide and then processed for TEM, as described above.

The percentage of lysosomes containing WGA (CG) or BSA (CG) was estimated by counting lysosomes with or without CG particles on ultrathin-sections (50 nm in thickness). CG particles on obliquely sectioned lysosomal membranes and in morphologically destroyed lysosomes were omitted from the counting.

**RESULTS**

WGA (CG)-binding experiments in RBC and their ghosts showed localization of CG particles on their surfaces, while in BSA (CG) reaction to them there were few CG particles that adhered closely to their membranes. Specific or nonspecific adsorption of BSA (CG) was negligible in their membranes (Table I). Most CG particles in the WGA reaction were distributed uniformly on the entire surface of the aldehyde-prefixed cell incubated at 37°C and on the unfixed cell surface incubated at 4°C (Fig. 1A). By contrast, an irregular distribution and many clusters of them were observed on the surface of unixed cells in the 37°C-incubation (Fig. 1B). In prefixed and unixed ghosts incubated with the labeled ligands in PBS (or the buffer 1) at 37°C, also, membrane-bound WGA (CG) indicated almost similar uniform and irregular distribution patterns on their outer surfaces, respectively (data not shown). In controls for the WGA (CG)-binding, the incubation with the labeled WGA in the presence of GlcNAc resulted in a failure to react (Fig. 1C).

WGA (CG)-bound RBC ghosts, where the membrane-bound WGA (CG) had been rearranged on their surface, were induced to yield small vesicles in the low ionic strength alkaline solution without bivalent cations at 37°C, as described by several workers in the intact ghosts (9, 20, 35). Budding of the membrane of ghosts into their interior and homogenization of their large vesicles by the 27 G needle could be confirmed in this treatment. Some of these vesicles included smaller single or plural vesicles and/or nonvesiculating membrane fragments in their lumen. CG particles were often found to be distributed irregularly on the inner surface of the vesicles and on the membrane fragments (Table II). Small amounts of particles were present on the outer surface of vesicles and in a free state far from the membranes. When these EDTA- and needle-treated ghosts were mixed with rat RBC and then incubated with anti-rat RBC antibodies, most CG particles in their 2,000 g-supernatant were seen on the inner surface of vesicles (Fig. 2A). The size of these vesicles was about 0.7 µm in diameter (0.7±0.4 µm). Under TEM, a fibrillar structure was identified on a little area of the outer surface in some GLA-tannic acid-fixed vesicles. This could not be found on the entire inner surface at all (Fig. 2B). By contrast, the fibrillar coat structure was clearly observed along the inner surface of the intact RBC ghost membrane prefixed by GLA-tannic acid (Fig. 2C).

In 27 G needle-homogenized ghosts in the buffer with MgSO₄ and BSA (CG), a large heterogeneity of vesicles was observed in their size and shape. The 37°C incubation after micropipette treatment of them resulted in for-

<table>
<thead>
<tr>
<th>Incubation</th>
<th>CG particles/µm</th>
<th>% in cluster</th>
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<tbody>
<tr>
<td><strong>Prefixed cells</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WGA (CG)</td>
<td>37°C</td>
<td>33.1±5.2</td>
</tr>
<tr>
<td>WGA (CG) + GlcNAc</td>
<td>37°C</td>
<td>&lt;1</td>
</tr>
<tr>
<td>BSA (CG)</td>
<td>37°C</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>Unfixed cells</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WGA (CG)</td>
<td>4°C</td>
<td>29.1±8.1</td>
</tr>
<tr>
<td></td>
<td>37°C</td>
<td>30.7±10.1</td>
</tr>
<tr>
<td>WGA (CG) + GlcNAc</td>
<td>37°C</td>
<td>&lt;1</td>
</tr>
<tr>
<td>BSA (CG)</td>
<td>4°C</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>37°C</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Aldehyde-prefixed or -unixed RBC was incubated with WGA (CG), WGA (CG) and GlcNAc, or BSA (CG) in PBS at 4°C or 37°C. Each result is given as the mean±S.D. of 60 determinations in 3 separate experiments.
Fig. 1.

Fig. 2.
Lysosome-Cell Membrane Vesicle Interaction

<table>
<thead>
<tr>
<th>Localization of WGA (CG)</th>
<th>Rates (%) before or after anti-RBC antibody treatment</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
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<tr>
<td>Vesicles Inner surface (+)</td>
<td>47.8±7.7</td>
</tr>
<tr>
<td>Outer surface (+)</td>
<td>7.2±2.8</td>
</tr>
<tr>
<td>Both surfaces (+)</td>
<td>0</td>
</tr>
<tr>
<td>Both surfaces (-)</td>
<td>14.9±5.0</td>
</tr>
<tr>
<td>Fragments (+)</td>
<td>13.9±5.4</td>
</tr>
<tr>
<td>(-)</td>
<td>16.2±6.1</td>
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</tbody>
</table>

RBC ghosts were treated with WGA (CG), EDTA (pH 8.0), and then anti-RBC antibody and RBC.

Table II. Proportion of vesicles and nonvesiculating fragments with or without WGA (CG) in WGA (CG)- and EDTA-treated ghosts.

Table III. Rates of vesicles and nonvesiculating fragments in ghosts homogenized in BSA (CG) and MgSO$_4$ solution.

<table>
<thead>
<tr>
<th>Localization of BSA (CG)</th>
<th>Rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesicles</td>
<td>60.4±9.1</td>
</tr>
<tr>
<td>Fragments</td>
<td>13.3±7.2</td>
</tr>
<tr>
<td>(+)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>(-)</td>
<td>25.7±9.7</td>
</tr>
</tbody>
</table>

A small amount of contaminants, e.g. mitochondria, were there in this preparation. Morphological features of these constituents were mostly typical electron-dense bodies. They displayed rounded, elongated or ring shaped structures. When the lysosome fraction was incubated with WGA (CG)-vesicles in the absence of cytosol, some parts of their limiting membranes at the adhering region were morphologically unclear or absent (Figs. 4B, 4C). In other contacting lysosome-vesicles, both limiting membranes at the adhering region were found in a partially collapsed state (Fig. 4D). In addition, some lysosomes included CG particles (Fig. 4E). The proportion (in number) of these IOV-connecting and CG-including lysosomes was markedly lower (about one third) of that in the interaction with WGA (CG)-vesicles (Table V). In the case of reaction of lysosomes with both vesicle types in the presence of supplemented ATP and its regenerating system, there was no definite difference in their ratios as compared with that in complementary experimental group without the additional ATP regenerating system.

Control experiments, where lysosome fractions were incubated with WGA (CG) or BSA (CG) alone, indicated very low rates of CG-containing vesicles under both conditions with and without supplemented ATP regenerating system (Table V). In these reactions, WGA

Fig. 1. Distribution pattern of WGA (CG) binding to unfixed RBC at 4°C (A and C) or 37°C (B) in PBS without (A and B) or with (C) GlcNAc. A; Many CG particles (arrowheads) were distributed on the 4°C-incubated cell surface. B; An irregular distribution pattern of the particles is observed on the cell surface in the 37°C incubation. Most particles form clusters (arrowheads). C; The particle is not found on the surface. Bars: 0.2 μm.

Fig. 2. Small vesicles (A and B) formed from RBC ghosts (C) by treatment with WGA (CG), EDTA and then a 27 G needle at pH 8.0. A; CG particles are located almost on the inner surface of the vesicles (arrows) and on the surface of membrane fragments (arrowheads), and scarcely on the vesicle’s outer surface. B; A high magnification photograph of aldehyde-tannic acid prefixed vesicles shows structure (arrowheads) on a little area of their outer surface. This fibrillar structure cannot be found on the outer surface (arrows) or on their entire inner surface. C; Aldehyde-tannic acid prefixed RBC ghost membranes. A fibrillar coat structure is observed on their inner surface (arrowheads). Bars: 0.1 μm.
(CG) was observed to bind to the surface of most lysosomes, but at most 9% of them included CG particles within them. Binding reactivity of BSA (CG) to the lysosome surface was almost negligible.

**DISCUSSION**

WGA, one of the lectins, binds specifically to GlcNAc and N-acetyl-neuraminic acid (27, 42). WGA (CG)-binding experiments demonstrate an irregular dis-
distribution pattern of WGA-binding sites on the surface of 37°C-incubated RBC, in contrast to the almost uniform pattern in 4°C-incubation. Results in the control with WGA inhibitor reveal that the WGA (CG)-binding is specific. These suggest induction of similar rearrangement of WGA-receptor complexes on the cell membrane to that of plasma membrane-bound multivalent ligands in early endocytic processes seen in other types of cells (10, 17, 18).

It has been known that intact RBC ghosts vesiculate spontaneously in a low ionic strength alkaline buffer without divalent cations to form small IOVs (19, 20, 31). EDTA-treatment (at pH 8.0) of WGA (CG)-bound ghosts under the same ionic strength also promotes inversion of their membrane to generate similar small vesicles, where CG particles were almost located along the inner surface of their limiting membrane. The present findings in WGA (Fer)-binding experiments suggest no WGA receptor on the outer surface of almost all these vesicles. The WGA has been reported to bind to sialic acids of glycoporphins on the outer surface of the RBC membrane (3). Sugar residues of glycoproteins and glycolipids in the normal cell membrane locate on the outer surface, but not on the inner aspect (13, 16). These morphological and biochemical criteria indicate that the formation of IOVs is also induced in the WGA (CG)-binding and EDTA-treated ghosts.

Steck et al. (31) have reported that the 27 G needle-homogenization of RBC ghosts in the presence of MgSO4 induced the generation of ROVs. However, these vesicles are markedly heterogeneous in their size and shape. The generation of small vesicles needs further homogenization through micropipettes. In these processes, BSA (CG) seems not to bind specifically to the membranes. Electron-microscopically, in the normal ghost, a fibrillar coat composed of submembranous cytoskeletal proteins, e.g. spectrins, are seen along the cytoplasmic surface (34, 35). The inner faces of most BSA (CG)-including vesicles, similarly, took on the cytoplasmic aspects of their parent ghost membrane. In addition, WGA (Fer)-binding experiments confirm the localization of WGA receptors on their outer surface. Thus, most of these small vesicles with BSA (CG) are evidently rightside-out.

The lysosomes have been well known to fuse with endosomes in vivo (2, 37). The present findings demonstrate that isolated liver lysosomes incorporate with small vesicles derived from RBC ghosts. The reactivity is higher to the IOVs with WGA (CG) bound to their inner surface than that to BSA (CG)-including ROVs. The markedly low reactivity to the latter vesicles resem-

### Table IV. WGA (Fer)-binding to outer surfaces of ghosts, WGA (CG)- and BSA (CG)-vesicles.

<table>
<thead>
<tr>
<th>Incubation</th>
<th>% of WGA (Fer)-binding</th>
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<tbody>
<tr>
<td></td>
<td>Ghosts</td>
</tr>
<tr>
<td>WGA (Fer)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>WGA (Fer)+ GlcNAc</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Values are given as means±S.D. (%) of 60 determinations from 3 experiments.

<sup>a</sup> Vesicles with WGA (CG) on their inner surface.

<sup>b</sup> BSA (CG)-including vesicles.

### Table V. Reaction of lysosomes to WGA (CG), BSA (CG), and WGA (CG)- or BSA (CG)-vesicles.

<table>
<thead>
<tr>
<th>Incubation</th>
<th>% of lysosomes with CG</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>− ATP</td>
</tr>
<tr>
<td>WGA (CG)</td>
<td>8.5±4.9</td>
</tr>
<tr>
<td>BSA (CG)</td>
<td>6.6±4.0</td>
</tr>
<tr>
<td>WGA (CG)-V</td>
<td>26.8±8.6</td>
</tr>
<tr>
<td>BSA (CG)-V</td>
<td>9.1±5.4</td>
</tr>
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</table>

The lysosomes were incubated with WGA (CG), BSA (CG), or WGA (CG)- or BSA (CG)-vesicle fraction [WGA (CG)-V, BSA (CG)-V] in the presence or absence of supplemented ATP regenerating system (+ ATP or − ATP). Results indicate means±S.D. of 90 determinations from 3 experiments.
bles that to WGA (CG) or BSA (CG) alone in the control experiments. Thus, it appears that the isolated liver lysosomes show a distinct interaction with EDTA-induced small IOVs originated from WGA (CG)-bound and -rearranged cell membranes.

In receptor-mediated endocytosis, it has been suggested that endocytosed ligands in coated vesicles are delivered to the endosomes after total or partial removal of the clathrin coat (14, 39) and then to the lysosomes after acidification of the endosomes (10, 14, 36). Liver lysosomes were reported to interact with late (dense) endosomes in a cell free system, but not with early (light) ones (24). On the other hand, our acellular system demonstrates that lysosomes can react with small IOVs generated from ligand-bound and -rearranged membranes under a low ionic strength alkaline buffer. Altstiel and Branton (1) described in another cell free system that bovine kidney lysosomes fused directly with coat-stripped vesicles, which had been isolated from the brain and then had their clathrins removed in an alkaline buffer. Detachment of submembranous cytoskeletal protein coat composed of spectrins and actins from the RBC ghost is also known to occur in the low-salt EDTA solution, accompanying IOV formation (9, 34). Our observation has confirmed no fibrillar coat structure on most outer surface areas of the IOVs. The coat depletion in the IOV outer surface may participate in its close adhesion to lysosomes. Furthermore, the collapse and disappearance of both limiting membranes of these IOVs and lysosomes at their adhering region suggest their fusion. In other several cell free systems, the fusion reaction between endocytic compartments seems to be energy- and cytosol-dependent (5, 12, 24, 38, 41). By contrast, in our present system, lysosome-IOV interaction differs from the other acellular systems in its cytosol dependency, though its requirement for an ATP regenerating system is unclear. Therefore, it is suggested that isolated liver lysosomes have a reactivity, at least to fuse, in cytosol-independent manner with the IOVs from ligand-receptor complex-rearranged and cytoskeletal protein-stripped RBC membranes.

Lysosomes do not react directly with the plasma membrane in normal cells. Little is understood about changes in membrane molecules during the endocytic pathway and about molecular events for lysosome-phagosome interactions. Recently, nonerythroid submembranous proteins and their binding proteins, i.e. spectrin-, ankyrin- and band 4.1-like molecules, were reported to play a role on the endocytosis and several other cellular functions (29, 30). Under these circumstances, our acellular system, using biochemicaly and morphologically well understood IOV membranes (34, 35) should be applicable for analyzing the interaction between lysosomes and intracellular membranes derived from the cell membrane.

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**REFERENCES**


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