PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **154**, 517-521 (1977)

Apparent Stabilization of Rat Liver Lysosomes by Cytosol and Serum Proteins[1] (39707)

B. EVAN BRENNER[2] AND JUDITH S. BOND[3]

Department of Microbiology and Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Lysosomes *in vivo* are surrounded by a cytoplasmic milieu which may affect the properties and functions of this organelle. The interactions between this subcellular particle and the cytoplasm are not well understood and need to be characterized in order to assess the physiological role of lysosomes. Interactions between lysosomes and cytoplasmic constituents might be detected by measuring the distribution of lysosomal enzymes in soluble and particulate compartments or fractions after incubation with various biochemicals. In such studies, lysosomes suspended in isotonic sucrose have been incubated with a large number of substances (1). Many biochemicals have been designated as labilizers (e.g., vitamin A) or stabilizers (e.g., cortisone) if they increased or decreased the distribution of lysosomal enzymes into the soluble fraction. The labilizers and stabilizers are thought to disrupt or protect, respectively, the lysosomal membrane. The present paper reports that rat liver-soluble proteins (cytosol), as well as serum proteins, cause soluble lysosomal enzymes to cosediment with cellular granules *in vitro*. Although these protein solutions appear to stabilize lysosomes, in actuality they enhance interactions between lysosomal enzymes and cellular particles by a mechanism other than stabilization of lysosomal membranes.

Materials and methods. Subcellular particles containing lysosomes were prepared from livers of male Holtzman rats (300 to 350 g) by homogenizing the sample in a tissue grinder with 0.25 *M* sucrose containing 0.01 *M* Tris-HCl buffer, pH 7.2, at a ratio of 1 g of liver:9 ml of sucrose solution. The centrifugation scheme for obtaining the large granular fraction (LGF), the fraction containing lysosomes, has been described by Weissmann (2). At each washing step, the LGF was suspended by directing a stream of buffered isotonic sucrose at the precipitate (3). The final protein concentration of the fraction was 2.4-4.0 mg/ml. To determine the total activity of β-glucuronidase, a sample of the large granular suspension was mixed with an equal volume of Triton X-100 [0.2% (v/v)], incubated at 37° for 30 min, and centrifuged at 20,000*g* for 20 min, and the supernatant fraction was assayed for enzyme activity.

To obtain the soluble contents (lysate) and sedimentable membranes of the LGF, the sedimented LGF was suspended and homogenized in distilled water and allowed to stand for 30 min at 4°. The osmotically shocked particles were then centrifuged at 48,000*g* for 80 min. The soluble portion (hypotonic lysate) was dialyzed against 1 m*M* Tris-HCl buffer, pH 7.2, for 12 hr at 4°; this portion contained 60-80% of the LGF β-glucuronidase activity. The sedimentable portion or membranes of the LGF, obtained after the hypotonic treatment, were homogenized into a buffered, isotonic sucrose solution.

For preparation of cytosol, liver samples were homogenized with 4 or 9 volumes of 0.25 *M* sucrose containing 0.01 *M* Tris-HCl, pH 7.2. Homogenates were centrifuged at 48,000*g* for 80 min and the supernatant (cytosol) fraction was decanted and stored at −20°. In some instances, livers were perfused with NaCl (0.9%) prior to homogenization and cytosol was dialyzed

---

[1] This work was supported in part by NSF Grant GB-44342 and NIH Grant CA-13715; B.E.B. had a Life of Virginia Fellowship.

[2] Present address: Department of Medicine, University Hospitals, Case Western Reserve University, Cleveland, Ohio 44106.

[3] Send reprint requests to Dr. Judith S. Bond, Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

ISSN 0037-9727

for 48 hr at 4° against the homogenizing buffer. To prepare boiled cytosol, cytosol was placed in a test tube and immersed in boiling water for 30 min. The flocculent precipitate was suspended by homogenization.

β-Glucuronidase activity was measured according to the method of Talalay *et al.* (4). Acid deoxyribonuclease activity was assayed according to the method of Gianetto and de Duve (5). Malic dehydrogenase activity was measured by the method of Siegel and Bing (6). Catalase activity was measured by the method of Beers and Sizer (7). The Biuret method (8) or the method of Lowry *et al.* (9) was used to determine protein concentrations. In both methods, bovine serum albumin (Fraction V) was used as the standard. Chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.), except as noted. Human γ-globulin fraction II was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio). Triton X-100 was purchased from Rohm and Haas (Philadelphia, Pa.).

Results. When suspensions of the LGF were incubated in isotonic sucrose, β-glucuronidase activity was solubilized with time (Fig. 1). After 60 min, approximately 25% of the total LGF β-glucuronidase activity had become nonsedimentable. However, in the presence of cytosol only ca. 6% of the total LGF β-glucuronidase activity became solubilized within 60 min. Cytosol alone contained some β-glucuronidase activity which was stable to incubation at 37° and nonsedimentable.

Control experiments demonstrated that β-glucuronidase activity was not inhibited by cytosol and that the activity was stable to incubation at 37°. The LGF activity, solubilized by Triton X-100, for example, was stable during incubation in sucrose or in cytosol (Fig. 1A). In addition, the measured activity of the cytosol + Triton-solubilized LGF was the sum of the parts. Solubilization of β-glucuronidase from the LGF was dependent on the concentration of cytosol incubated with the LGF in the range of 3 to 16 mg of cytosol protein/ml of incubation mixture.

Dialyzed or boiled cytosol had the same effect as untreated cytosol on the solubilization of β-glucuronidase from the LGF (Fig. 2). No significant differences were observed between the effects of boiled cytosol, subsequently dialyzed or dialyzed cytosol that was subsequently boiled. Boiling destroyed β-glucuronidase activity in the cytosol as well as other enzymatic activities (e.g., catalase and malic dehydrogenase). Boiled cytosol did not affect the solubilization of catalase, a peroxisomal enzyme, or malic dehydrogenase, a mitochondrial enzyme (Fig. 2). Experiments with acid deoxyribonuclease and acid phosphatase (not shown), two lyso-

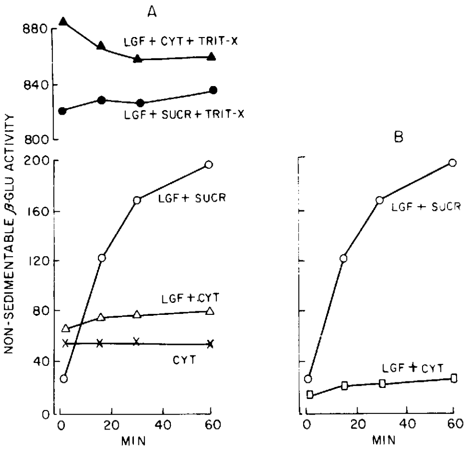

FIG. 1. Effect of sucrose or cytosol on the solubilization of β-glucuronidase of the large granular fraction (LGF). The LGF (containing 22 mg of protein/tube) was suspended in either isotonic sucrose or cytosol (15 mg of protein/ml) in a total volume of 7.5 ml. The LGF suspensions were incubated, with shaking, at 37°, pH 7.2. Samples (0.5 ml) were either centrifuged directly at 40,000*g* for 10 min or mixed with Triton X-100 and subsequently centrifuged. β-Glucuronidase activity is expressed in μg of phenolphthalein formed/30 min/500 μl of sample. A. Nonsedimentable β-glucuronidase activity of the LGF incubated with sucrose (○) or with cytosol (△) or of the cytosol alone (×) in buffered isotonic sucrose; total activity of the LGF in sucrose (●) or in cytosol (▲) was also determined. B. Similar to A except that the activity in the cytosol alone (×) has been subtracted from the measured nonsedimentable LGF plus cytosol activity (△) to yield the net difference of the mixture (□). This is a representative experiment selected from 12 replications. For the LGF incubated with sucrose, after 60 min, 22 ± 1% (mean ± standard error of the mean) of the total β-glucuronidase activity had become nonsedimentable. In the presence of cytosol, 6.0 ± 0.6% of the total LGF β-glucuronidase activity was nonsedimentable after 60 min of incubation.

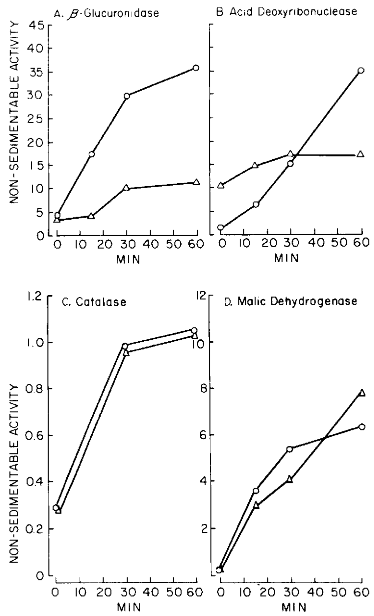

FIG. 2. Effect of boiled cytosol on the solubilization of enzymes from the LGF. The LGF was suspended in isotonic sucrose (O) or in boiled cytosol (Δ) (16 mg of protein/ml) and incubated at 37°. A. β-Glucuronidase is expressed in μg of phenolphthalein formed 30 min^{-1} 500 μl^{-1} of sample; B. Acid deoxyribonuclease is expressed in μg of deoxyribonucleotides formed 30 min^{-1} 500 μl^{-1} of sample; C. Units of catalase were k min^{-1} values (first -order rate constant) 50 μl^{-1} sample; and D. Units of malic dehydrogenase were μmole of β-NADH consumed min^{-1} 10 μl^{-1} sample. These are representative experiments. For β-glucuronidase, five additional experiments were conducted; for each of the three other enzymes, three additional experiments were conducted.

somal enzymes, indicated that solubilization of these enzymes was depressed by cytosol.

Cytosol added to the LGF 2 min prior to centrifugation was just as effective in causing β-glucuronidase activity to sediment as cytosol present continuously during the 37° incubation (Fig. 3). In contrast, cortisone acetate retarded the release of the enzyme if present during incubation but did not cause coprecipitation of β-glucuronidase when added to the LGF incubation mixture 2 min before centrifugation.

Soluble β-glucuronidase activity in the cytosol or derived from a hypotonic lysate of the LGF and then mixed with cytosol, sedimented in the presence, but not the absence, of the LGF membranes (Table I). Thus coprecipitation of β-glucuronidase activity was only observed when cytosol and LGF membranes were both present in the incubation mixture.

Albumin and γ-globulins also reduced the solubilization of β-glucuronidase whether added during incubation of the LGF or 2 min before centrifugation of the LGF preincubated in sucrose (Fig. 4). These serum proteins did not inhibit β-glucuronidase activity. In other experiments, RNA (25 mg/ml of isotonic sucrose) and heparin (25 mg/ml of isotonic sucrose), had no effect on the solubilization of β-glucuronidase at neutral pH. In the experiments described heretofore, the pH of the incubation mixture was pH 7.2. When the LGF was incubated at pH

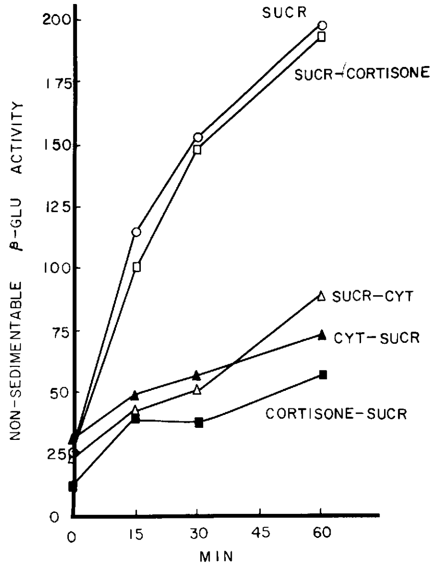

FIG. 3. Effects of cytosol and cortisone on the solubilization of β-glucuronidase activity. The LGF was suspended in isotonic sucrose (O), cytosol, 28 mg of protein/ml (▲), or cortisone acetate, 1.25 mg/ml of isotonic sucrose plus 1% dimethyl sulfoxide (DMSO) (■). Samples were removed from the cytosol (▲) and cortisone (■) suspensions, mixed with an equal volume of isotonic sucrose, incubated for an additional 2 min at 37°, and then centrifuged. Samples were also removed from the sucrose suspension at various intervals and mixed with an equal volume of isotonic sucrose (O), cytosol (Δ), or cortisone acetate in isotonic sucrose plus 1% DMSO (□); these samples were then incubated for an additional 2 min before centrifugation. The β-glucuronidase activity in the cytosol alone has been subtracted. DMSO (1%) alone did not affect the solubilization of β-glucuronidase. This is a representative experiment selected from three replications.

TABLE I. EFFECT OF THE HYPOTONIC SEDIMENT (MEMBRANES) OF THE LARGE GRANULAR FRACTION (LGF) ON SEDIMENTATION OF β-GLUCURONIDASE ACTIVITY[a].

	β-Glucuronidase activity		
		After centrifugation	
Fractions	Prior to centrifugation	Soluble	Sedimentable
Lysate	60	58	1
Membranes	41	29	10
Cytosol	48	41	6
Lysate and cytosol	100	97	5
Membranes and cytosol	88	32	53
Membranes and lysate	100	84	13
Membranes and lysate and cytosol	140	53	96

[a] The lysate (hypotonic lysate of the LGF) and membranes (hypotonic sediment of the LGF) were prepared as described in the materials and methods section and were diluted 20-fold prior to use. All tubes contained 1 ml of lysate, membranes, or cytosol, or mixtures of these solutions. The volumes of all mixtures were adjusted to 3.0 ml with 0.01 *M* Tris-HCl buffer, pH 7.2. The mixtures were incubated at 37° for 2 min. These experiments were repeated three times with different LGF preparations and the results were similar to those shown above.

5, cytosol or albumin did not sediment β-glucuronidase activity.

Discussion. In many studies on lysosomal stabilization *in vitro* it is assumed that the lysosomal membrane is stabilized when a biochemical agent appears to prevent the solubilization of a lysosomal enzyme and there is less leakage or release of the activity into the medium. On the basis of the results of the present work, this assumption warrants reexamination, for it is not justified under certain conditions. It has been demonstrated that β-glucuronidase cosediments with the LGF at pH 7 in the presence of cytosol, boiled cytosol, albumin, and γ-globulin. Thus, while these agents would *appear* to be lysosomal stabilizers, more likely they cause a coprecipitation of soluble lysosomal enzymes with cellular particles rather than stabilization of the membrane per se. These results are in contrast to those with cortisone acetate which truly is able to stabilize lysosomes.

The cosedimentation of lysosomal enzymes with the LGF in the presence of cytosol has some specificity, because a mitochondrial enzyme and a peroxisomal enzyme are not coprecipitated. Thus, cytosol may foster a selective interaction between the LGF membranes and specific enzymes.

The findings in I-cell disease (10), which indicate that lysosomal enzymes have a natural affinity for lysosomes, are pertinent to this work. Cytosol and serum proteins may be instrumental in promoting the interaction of lysosomal enzymes and lysosomes.

Other researchers have incubated protein solutions with lysosomes at pH 7. These protein solutions have been sera from (a) pregnant patients (11), (b) patients with chronic hepatitis and systemic lupus erythematosus (12), and (c) rabbits immunized against rat polymorphonuclear leukocyte membranes (13). These workers attribute

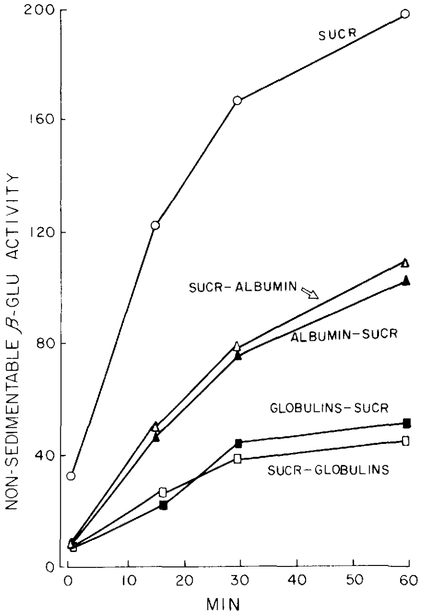

FIG. 4. Effect of albumin and γ-globulins on the solubilization of β-glucuronidase activity of the LGF. The LGF was suspended in either isotonic sucrose, an albumin solution, 23 mg/ml of isotonic sucrose (▲) or a γ-globulin solution, 22 mg/ml of isotonic sucrose (■). Samples were removed from the isotonic sucrose suspension, mixed with an equal volume of isotonic sucrose (○), the albumin solution (△), or the γ-globulin solution (□), and incubated for 2 min at 37°. Samples also were removed from the albumin suspension (▲) or the γ-globulin suspension (■), mixed with an equal volume of isotonic sucrose, and incubated for 2 min at 37°. These results were found consistently in four similar experiments.

the lower nonsedimentable lysosomal enzyme activity in the presence of certain serum samples to their stabilizing effects on the lysosomal membrane. Although test sera were compared to normal sera, it is quite possible that selective effects are due to cosedimentation of the lysosomal enzymes by components of the test sera.

The cosedimentation of lysosomal enzyme activity requires rather specific conditions; it occurs at pH 7 but not at pH 5 and the hypotonic sediment of the LGF must be present. The fact that boiled cytosol is able to affect cosedimentation of lysosomal enzymes indicates that subtle surface properties of proteins are not responsible for the interaction. On the other hand, the consistent finding that γ-globulins were more effective than albumin indicates that certain physical properties of the proteins in the LGF environment are determinative factors in cosedimentation. Important factors in this regard may be the net charge of a protein or the state of glycosylation of the protein. These observations raise the intriguing possibility that proteins having particular physicochemical properties may interact preferentially *in vivo* with lysosomes or other subcellular particles and that this may be determinative in the heterogeneity of half-lives of soluble intracellular enzymes.

Summary. Cytosol or serum proteins promote the cosedimentation of rat liver lysosomal enzymes with the large granular fraction membranes at neutral pH. Cytosol promoted cosedimentation of three lysosomal enzymes but not a mitochondrial or a peroxisomal enzyme. Dialyzed or boiled cytosol are as effective as untreated cytosol whereas γ-globulin is more effective than albumin in promoting cosedimentation.

---

1. Weissmann, G., Fed. Proc. **23,** 1038 (1964).
2. Weissmann, G., Biochem. Pharmacol. **14,** 525 (1965).
3. Shibko, S., and Tappel, A. L., Biochem. J. **95,** 731 (1965).
4. Talalay, P., Fishman, W. H., and Huggins, C., J. Biol. Chem. **166,** 757 (1946).
5. Gianetto, R., and de Duve, C., Biochem. J. **59,** 433 (1955).
6. Siegel, A., and Bing, J., Proc. Soc. Exp. Biol. Med. **91,** 604 (1956).
7. Beers, R. F., and Sizer, I. W., J. Biol. Chem. **195,** 133 (1952).
8. Gornall, A. G., Bardawill, J., and David, M. M., J. Biol. Chem. **177,** 751 (1949).
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
10. Hickman, S., and Neufeld, E. F., Biochem. Biophys. Res. Commun. **49,** 992 (1972).
11. Hempel, K. H., Fernadez, L. A., and Persellin, R. H., Nature (London) **225,** 955 (1970).
12. Wiedermann, G., Hirschhorn, R., Weissmann, G., and Miescher, P. A., *in* "Fourth International Symposium on Immunopathology" (P. Grabar and P. A. Miescher, eds.), Grune and Stratton, New York (1965).
13. Persellin, R. H., J. Immunol. **103,** 39 (1969).

Received September 1, 1976. P.S.E.B.M. 1977, Vol. 154.