STUDIES ON THE RELEASE OF LYSOSOMAL ENZYMES FROM KIDNEY LYSOSOMES

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ABSTRACT

Incubation of kidney lysosomes at 37° results in a graded release of lysosomal enzymes. The release of enzyme occurs in two stages. First the enzymes become available to the substrate but remain sedimentable. Later the amount of soluble enzyme increases and eventually is almost equal to that of the available enzyme. Morphological studies of lysosomes showed that during the process involving increasing availability of enzymes, the lysosomes remained intact. Release of the soluble enzymes was characterized ultrastructurally by a complete loss of the electron-opaque matrix contained within the lysosomal membrane. The increased release of soluble enzymes was concomitant with an increase in the number of individual lysosomes showing complete loss of contents, rather than a gradual loss or dilution of matrix density. Lysosomes which had lost their electron-opaque contents retained their outer membrane intact and were seen to contain numerous internal membranes and small vesicles.

INTRODUCTION

The work of de Duve and his associates (1) resulted in the concept of lysosomes as membrane-limited cytoplasmic particles containing hydrolytic enzymes. This has been further supported by morphological studies of liver (2) and kidney (3), which have shown that particles marked by a histochemical acid phosphatase reaction were surrounded by a single outer membrane. Since lysosomes are characterized by their latent enzyme activity, changes in their structure may be expected to occur during the release of enzymes. Kidney lysosomes incubated at 37° show a graded release of enzymes (4) and provide a suitable system for studying structural changes. The work reported here describes the biochemical and morphological changes that occurred when suspension of purified kidney lysosomes were subjected to this treatment.

MATERIALS AND METHODS

Tissue Fractionation

Lysosomes were prepared from the kidneys of male Sprague-Dawley rats (250 to 350 gm) by methods previously described (4).

Enzyme Assays

Acid phosphatase and arylsulfatase were chosen as typical lysosomal enzymes for this study. Acid phosphatase was measured by the method of Gianetto and de Duve (5) using sodium β-glycerophosphate as substrate. The released phosphate was measured by the method of Lowry *et al.* (6). Arylsulfatase was measured by the method of Roy (7) using 2-hydroxynitrophenyl sulfate as substrate. Protein was determined by the method of Miller (8).

GRADED RELEASE OF ENZYMES FROM INTACT KIDNEY LYSOSOMES: Suspensions of freshly pre-

pared lysosomes in 0.6 M sucrose containing 0.34 mM ethylenediaminetetraacetate (EDTA) pH 7 were incubated at 37°. At appropriate time intervals the available, the soluble and the total acid phosphatase and arylsulphatase activities, soluble protein and optical density (light scattering) at 540 mμ were determined. The available enzyme activity was determined by incubating the lysosomes for 10 minutes at 37° in substrate containing 0.6 M sucrose. For determination of the soluble enzyme, the lysosomal suspension was centrifuged for 10 minutes at 15,000 *g* and the enzyme activity of the supernatant solution measured. The supernatant solution was also used for measurement of the soluble protein. Total enzyme activity was determined after freezing and thawing the suspension ten times.

ELECTRON MICROSCOPY OF LYSOSOMES DURING RELEASE STUDIES: At the time intervals when aliquots of the lysosomal suspension were removed for enzyme analysis, an additional sample was removed for morphological examination. The suspension was centrifuged for 10 minutes at 15,000 *g* and the pellet fixed for 2 hours with 1 per cent buffered osmium tetroxide containing 0.6 M sucrose. The pellets were dehydrated in alcohol and embedded in epoxy resins, and thin sections were cut on a Porter-Blum microtome with glass or diamond knives and examined with an RCA EMU-3 microscope. Lysosomes that had been disrupted by freezing and thawing ten times were centrifuged for 60 minutes at 100,000 *g* in order to obtain a pellet for these studies.

RESULTS

Release of Lysosomal Enzymes

Fig. 1 shows the changes that occurred when suspensions of freshly prepared lysosomes were incubated at 37°. At the commencement of the incubation period, the enzymes were found to be latent to the extent of 90 to 95 per cent. Incubation resulted in a decrease in optical density which corresponded to an increase in the availability of the enzymes, with only small amounts of the enzymes and protein becoming soluble. These early stages were followed by a more rapid release of soluble enzymes and protein and at the end of the experimental period (3 hours) the amount of soluble enzyme was nearly equal to that of the total available enzyme. Fifteen to 20 per cent of the total enzymes remained sedimentable with the lysosomal membrane fraction which also contained 20 per cent of the total protein.

When the lysosomal suspensions were frozen and thawed ten times, the enzymes became completely available. However, only 80 per cent of the enzymes were soluble, the remainder being associated with the membranous material (Table I).

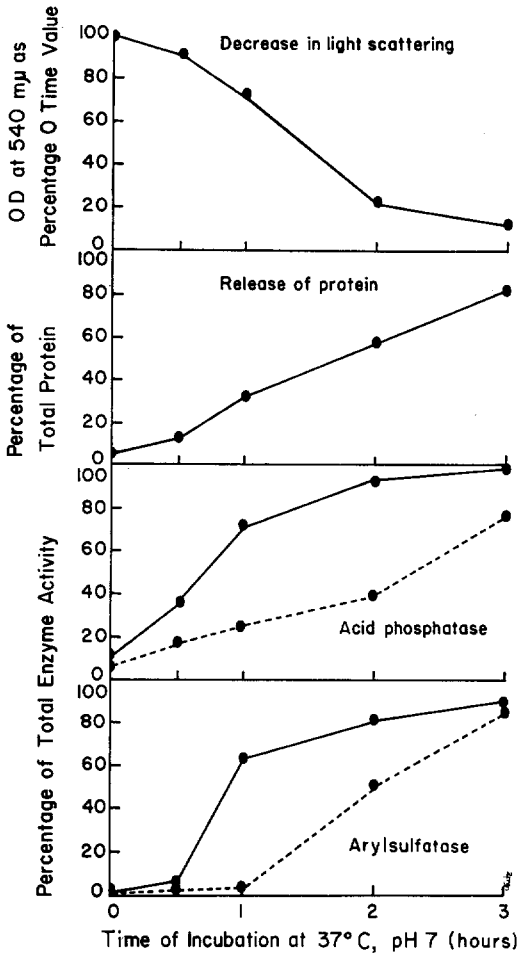

FIGURE 1 Availability and release of lysosomal enzymes and protein from kidney lysosomes suspended in 0.6 M sucrose containing 0.34 mM EDTA buffer pH 7 and incubated at 37°. Changes in light scattering properties of suspension are also included. The results are expressed as percentage of total activity at 0 time. Solid lines (——) represent enzyme available, broken lines (----) enzyme released in soluble form.

Morphological Changes Occurring During Release of Lysosomal Enzymes

Fig. 2 shows the lysosomal preparation at 0 time of incubation. The preparation consists mainly of intact lysosomes which can be clearly

TABLE I

Release of Lysosomal Enzymes by Freezing and Thawing

Rat kidney lysosomes were frozen and thawed 10 times and then centrifuged at 100,000 *g* for 60 minutes. Results represent percentage of total enzyme activity found in soluble and particulate fraction.

	Insoluble fraction	Soluble fraction
Protein	18	82
Acid phosphatase	48	52
Arylsulfatase	46	54

distinguished from the small amount of mitochondrial contamination present. The lysosomes contain an electron-opaque matrix, surrounded by a clearly defined membrane. No internal structure can be distinguished.

After 3 hours incubation at 37°, the lysosomes are essentially of the same size as at 0 time but have lost their opaque matrix and are bounded by a single membrane structure which encloses numerous small vesicles and other membranous material (Fig. 3). More complete disruption of the lysosomes also occurs, giving rise to the numerous external vesicles also present.

Sections of the pellets after 30 minutes and 1 hour did not appear to be significantly different from those at the commencement of the experiment, although a considerable amount of the total enzyme had become available but not soluble. After 2 hours, when the amount of soluble enzyme had greatly increased, sections showed that the lysosomes were intact as in Fig. 2 or had lost the electron-opaque matrix as in Fig. 3. Lysosomes that had lost part of their contents were rarely seen. Because the intact lysosomes and the lysosomes that had lost their contents separated into two distinct layers during centrifugation procedure for preparing pellets, sections at this stage appeared either as those shown in Fig. 2 or as in Fig. 3.

When lysosomes were frozen and thawed, complete disruption of the particles occurred (Fig. 4). The sedimentable fraction obtained after this treatment revealed extensively fragmented membrane material and small vesicles. Larger amounts of enzyme remain associated with fractions prepared in this manner, although the total amount of protein released is almost the same as that released when the lysosomes were incubated at 37° (Table I). As the lysosomal enzymes may only represent a small percentage of the total lysosomal proteins, the differences in the amount of enzyme released by these treatments may indicate either that a different release mechanism exists or that less non-specific binding of enzymes to the lysosomal membrane occurs when lysosomes are incubated at 37°.

DISCUSSION

Studies of the release of enzymes from intact lysosomes by biochemical techniques indicate that this process occurs in two stages. First, there is an increase in the availability of enzymes but little release, and then at a later stage availability and release become essentially equal. This suggests that the first stage represents a change in the permeability of the lysosomal membrane, permitting entry of substrate but not loss of enzymes. It is not known whether this stage is reversible. Morphological studies of the lysosomes during this period showed little structural change. The second stage was characterized by a release of enzymes from the sedimentable fraction which electron microscopy showed to consist almost completely of lysosome membranes. At no stage during the graded release of enzymes did lysosomes appear to lose only part of their electron-opaque matrix. Loss of the matrix always appeared to be complete. These results suggest that the different stages in the release of enzymes do not represent partial loss of contents from all the lysosomes, but an increase in the number of lysosomes that have lost their matrix completely.

The appearance of numerous small vesicles and other membranous material after loss of the electron-opaque matrix is of interest as it may represent part of a complex internal structure. Studies by Sabatini *et al.* (9) and Miller (10) on intact kidney lysosomes have suggested some internal structure. Furthermore, the reabsorption of hemoglobin by kidney tubule cells with its subsequent digestion is characterized by the appearance of numerous whorls of membranous material (10), similar to those observed in mouse liver lysosomes (11). The internal membrane in these particles did not appear to be derived from residual membranous structure of mitochondria and other organelles undergoing digestion as seen in cytolysomes (12) although Miller has also shown

that these conditions can give rise to kidney lysosomes containing mitochondrial structures (13). The kidney lysosomes used in these experiments are probably derived from protein absorption droplets formed during stimulation of reabsorption (14). However, no mitochondrial structures were observed in these lysosomes. Further, internal membranous structures not derived from other subcellular particles have also been observed in the cytosomes and multivesicular bodies of liver (15). In the case of kidney, Miller (10) suggested that these membranes may be formed from phospholipids entering the protein droplets at the time of protein reabsorption and subsequently associating with some of the protein to form characteristic phospholipid membranes. We conclude from our study that the pieces of membranes and small vesicles found inside the disrupted lysosome represent the remains of an internal membrane structure. At present the role of these internal membranes is not known, nor is there any information on any enzyme activity they may possess other than that of the lysosomal enzymes that remain associated with them presumably by non-specific adsorption or mechanical entrapment.

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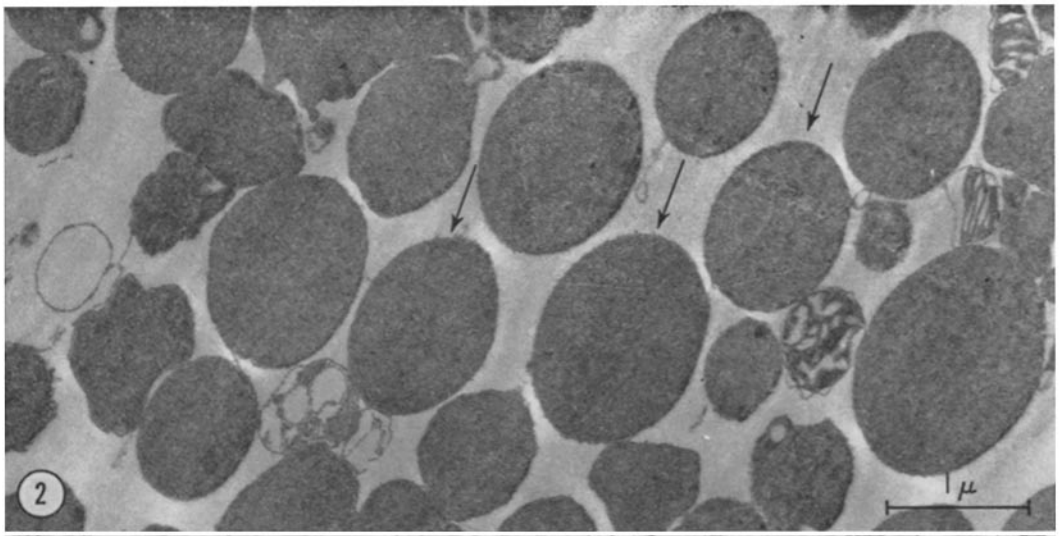
FIGURE 2 Intact kidney lysosomes at commencement of incubation period. The membrane surrounding the lysosomes can be observed (arrows). A small amount of mitochondrial contamination can be seen. × 18,000.

FIGURE 3 Kidney lysosomes after 3 hours incubation at 37°. Lysosomes show complete loss of electron-opaque matrix, but the outer membrane has retained the shape and size of the undisrupted lysosome. Membranous struc tures and numerous small vesicles are present within the limiting membrane (arrows). × 18,000.

FIGURE 4 Membrane fraction obtained by centrifuging preparations of lysosomes that have been disrupted by freezing and thawing ten times. × 18,000.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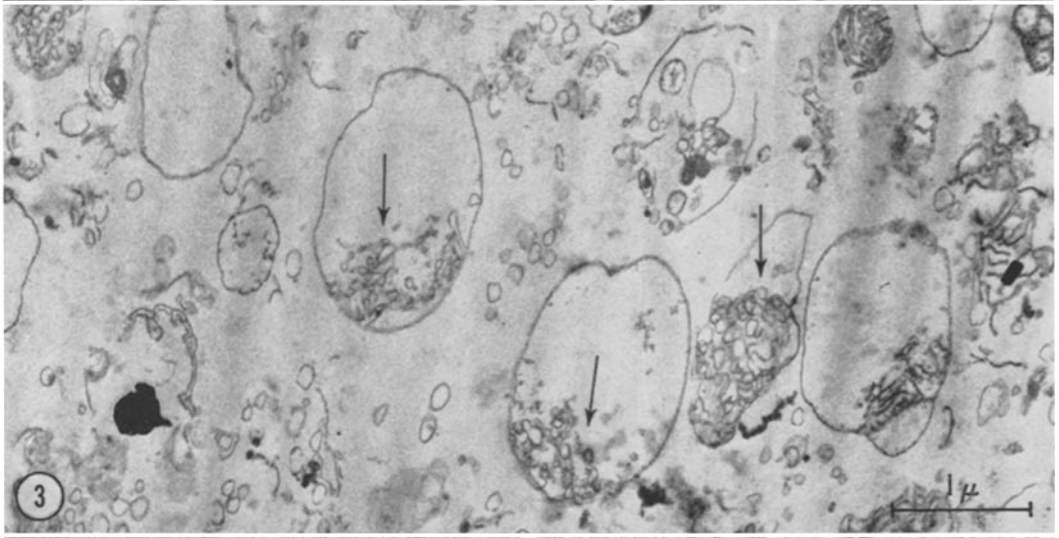
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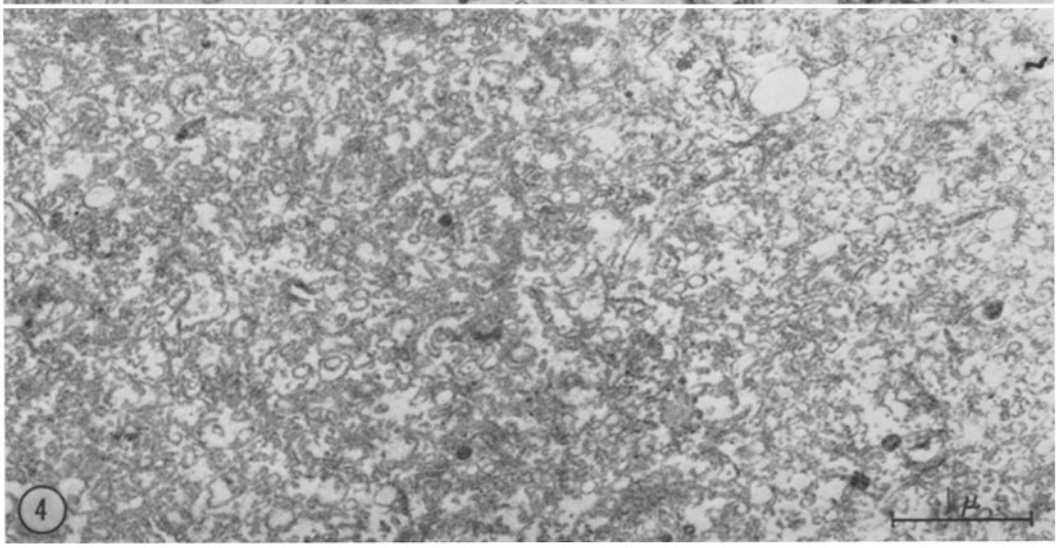
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