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TECHNICAL NOTE

A simple procedure for the isolation of rat kidney lysosomes

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A simple procedure for the isolation of rat kidney lysosomes.

Background. A procedure for the isolation of highly purified lysosomes from normal rat kidney is described.

Methods. The method depends on the swelling of mitochondria when the postnuclear supernatant fraction is incubated with 2 mM Ca^{2+}. The lysosomes can then be separated from the swollen mitochondria by Percoll density gradient centrifugation.

Results. The lysosomal fraction obtained by our method was enriched more than 30-fold in terms of marker enzymes with a yield of about 11%. Electron microscopic examination and the measurement of the activities of marker enzymes for various subcellular organelles indicated that our lysosomal preparation was essentially free from contamination by other organelles.

Conclusion. We believe that this procedure for isolating kidney lysosomes will be useful in the study of the mechanisms of specific modification, processing and catabolism of proteins.

The role of kidney lysosomes in the accumulation and catabolism of "exogenous" proteins has been established by work from many laboratories [1–7]. Lysosomes contain many hydrolytic enzymes, which have been shown to digest a wide variety of proteins. Furthermore, these enzymes are distributed in all sorts of membranes, and can be associated with the specific modification, processing, and catabolism of membrane proteins.

The isolation of lysosomes by conventional differential centrifugation is extremely difficult. As extensively discussed by Beaufay [8], the difficulty lies in the fact that there are only minor differences in the isopyknic density or sedimentation coefficient among lysosomes, mitochondria, and peroxisomes.

We previously described a procedure used for the isolation of rat liver lysosomes [9]. In the present study, we isolated kidney lysosomes from homogenates of rat kidney cortex by a procedure in which the isopyknic properties of the mitochondria, rather than the lysosomes, are altered. The procedure is based on the swelling of mitochondria in the presence of Ca^{2+} [10], where the swollen mitochondria can be separated from the lysosomes by Percoll density gradient centrifugation.

Key words: membrane proteins, lysosomes, enzymes, catabolism.

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METHODS

Tissue fractionation

Male anesthetized Wistar rats, 200 g body wt, were starved for 18 hours and killed by decapitation. The kidneys were rapidly removed and the renal cortex was cut with scissors. The cortical tissue was immediately weighed, minced with scissors, and suspended in 6 volumes of cold 0.3 M sucrose bubbled with nitrogen gas and containing 50 μg/ml leupeptin. It was homogenized in a six-stroke Potter-Elvhjem homogenizer with a Teflon pestle rotating at 1000 rpm. The homogenate was centrifuged at 1000 rpm for five minutes and the supernatant was centrifuged at 2000 rpm for five minutes. In this article the supernatant obtained after the second centrifugation is named the postnuclear supernatant.

Isolation of lysosomes

The postnuclear supernatant was incubated for 10 minutes at 37°C in the presence of 2 mM $CaCl_2$ to induce mitochondrial swelling [10]. The incubated sample was layered on an iso-osmotic Percoll at a density of 1.23 g/ml in a Hitachi RP 30 rotor tube. The pH of the Percoll suspension was adjusted to 7.4 with hydrochloric acid. Centrifugation was performed for 15 minutes at 60,000 × g in a Hitachi RP 30 rotor at 2°C. After centrifugation, fractions of 2.5 ml were collected from the bottom of the tube and diluted with 5 volumes of 0.3 M sucrose. The solution was subsequently centrifuged at 15,000 rpm for 10 minutes to obtain a pellet. The pellet was washed twice by centrifugation under the same conditions, which effectively removed the contaminating Percoll. The washed pellet was finally suspended in a small volume of 0.3 M sucrose and was used as the purified lysosomal fraction.

Enzyme assays

Cathepsin D [11] and arylsulfatase (combined activities of A and B) [12] used as lysosomal markers. Succinic-INT

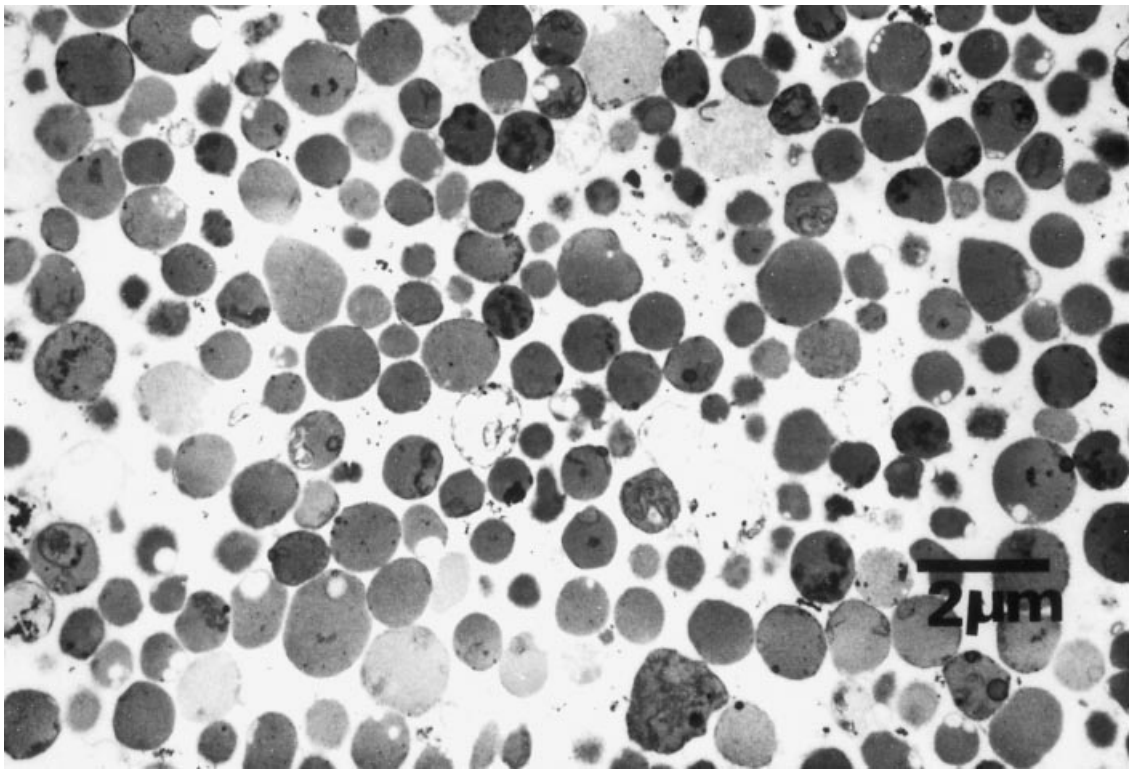

Fig. 1. Electron micrograph of the purified lysosomal fraction.

reductase [13], glucose-6-phosphatase [14], and catalase [15] were used as mitochondrial, microsomal, and peroxisomal markers, respectively.

Protein determination

Protein was determined using the BCA Protein Assay Reagent (Pierce) [16].

Morphological examination

The final pellet, prepared as described above in the section on isolation of lysosomes, was fixed with 2.5% glutaraldehyde for two hours at 0°C. After fixation, the sample was washed several times with 0.25 M sucrose solution buffered with 50 mM cacodylate (pH 7.4) and it was then post-fixed with 1% osmium tetroxide in 0.25 M sucrose buffered with 0.1 M cacodylate (pH 7.4) for one hour at 0°C. The sample was subsequently dehydrated with ethanol, and embedded in Epoxyresin. Sections were stained with 2% uranyl acetate for 50 minutes, followed by lead citrate for 20 minutes. They were then examined with a Hitachi H-500 electron microscope.

RESULTS

Morphological examination

As seen by electron microscopy, the purified lysosomal fraction consisted almost exclusively of membrane-limited granules approximately 0.5 to 1.5 μm in diameter, which revealed a morphological heterogeneity; some were round and electron-dense while others were vacuolated (Fig. 1). This heterogeneity was in agreement with numerous cytochemical observations *in situ* that show the various forms acid phosphatase-positive structures can take. Contamination by other organelles was not observed.

Table 1. Purification of rat kidney lysosomes by Percoll density gradient centrifugation

Enzyme	Enzyme specific actvity *units/mg protein*	Relative specific activity *lysosome/homogenate*	Yield *%*
Cathepsin D	636	30.3	12
Arylsulfatase	1400	32.6	10
Catalase	151	0.030	—
Succinic INT reductase	ND	—	—
Glucose-6-phosphatase	ND	—	—

Relative specific activity are expressed as specific activity found in the purified lysosme/specific activity measured in the homogenate.
Values are mean of seven experiments.

Biochemical analysis

Table 1 shows the yield and the relative specific activity of marker enzymes for various cell organelles in the purified lysosomal fraction. The purified lysosomal fractions were very rich in both cathepsin D and arylsulfatase, more than 30-fold over the homogenate. Succinic-INT reductase and glucose-6-phosphatase were below the level of detection. We detected a slight activity of catalase in our

lysosome preparation. However, if one assumes that the protein content of peroxisomes was comparable to that of lysosomes, the relative specific activity of 0.030 for catalase as compared to more than 30 for lysosomal marker enzymes would indicate that the contamination by peroxisomes constitutes less than 0.1% of the lysosomal proteins. The lysosomal fraction contained about 12 and 10% of cathepsin D and arylsulfatase of the homogenate, respectively.

DISCUSSION

In the present study, we isolated lysosomes from the renal cortex. An effective separation of lysosomes from mitochondria and peroxisomes by isopyknic centrifugation is possible only if the density of lysosomes has been selectively changed by injecting the animal with appropriate substances that enter the lysosomes by phagocytosis. Thus, methods have been described for isolating lysosomes filled with Dextran 500 [8], Triton WR-1339 [17], and colloidal iron [18]. The great advantage of our procedure is that it does not require preliminary treatment of the animals, and thus, lysosomes are not rendered "abnormal" by overloading with foreign materials. Our procedure is based on the swelling of mitochondria in the presence of Ca^{2+} [10]. The swollen mitochondria can be separated from lysosomes by Percoll density gradient centrifugation. As seen in Figure 1, the lysosomes obtained from rat kidney cortex are approximately 0.5 to 1.5 μm in diameter, and as such have an identical ultrastructure to the type of lysosomes most frequently observed in sections of proximal tubule cells [19]. As seen in Table 1, in the purified kidney lysosomal fractions, the relative specific activity of more than 30 for lysosomal marker enzymes is the highest ever reported in any lysosomal preparation. The biochemical observations were in good agreement with the electron microscopic observations and demonstrated a very high degree of purification of kidney lysosomes.

As originally reported by de Duve et al, lysosomes are organelles that have many hydrolytic enzymes that can digest a wide variety of proteins, and that play an important role in heterophagy and autophagy [20]. Furthermore, these enzymes are distributed in all sorts of membranes, and can be associated with the specific modification, processing, and catabolism of membrane proteins [21, 22]. Buus, Sette and Grey demonstrated that antigen proteins should be treated with hydrolytic enzymes in lysosomes to present antigenicity on the surface of T cells [23]. The role of kidney lysosomes in the accumulation and catabolism of "exogenous" proteins has been established by worker in several laboratories [1–7]. The kidney lysosomal fraction was about 2.5-fold richer than the liver lysosomal fraction in terms of marker enzymes as compared in our previous study (data not shown), suggesting that the kidney plays an important role in the metabolism of several proteins. Figure 2 shows the result of Western blot analysis of the kidney lysosomal albumin in a nephrotic rat. Albumin is indicated to be physiologically degraded in kidney lysosomes, and this degradation is increased in nephrotic syndrome.

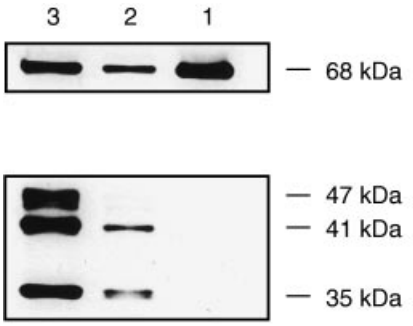

Fig. 2. Western blot analysis of kidney lysosomal albumin. Total lysosomal proteins of kidney cortex from rats treated (lane 3) and non-treated (lane 2) with puromycin aminonucleoside (10 mg/100 g body wt) by intraperitoneal injection, and rat serum (lane 1) were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellurose sheets, and reacted with anti-albumin serum, followed by horseradish-peroxidase anti-rabbit IgG.

We hope that our procedure for the isolation of kidney lysosomes will be able to clarify the mechanism of the specific modification, processing, and catabolism of several proteins.

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REFERENCES

1. Straus W: Isolation and biochemical properties of droplets from the cells of rat kidney. *J Biol Chem* 207:745–755, 1954
2. Carone FA, Peterson DR, Oparil S, Pullman TN: Renal tubular transport and catabolism of proteins and peptides. *Kidney Int* 16:271–278, 1979
3. Sumpio BE, Hayslett JP: Renal handling of proteins in normal and disease states. *Quart J Med* 57:611–635, 1985
4. Maack T, Johnson V, Kau ST, Figueiredo J, Sigulem D: Renal filtration, transport, and metabolism of low-molecular-weight proteins. *Kidney Int* 16:251–270, 1979
5. Christensen EI: Rapid protein uptake and digestion in proximal tubule lysosomes. *Kidney Int* 10:301–310, 1976
6. Camargo MJF, Sumpio BE, Maack T: Renal hydrolysis of absorbed protein: Influence of load and lysosomal pH. *Am J Physiol* 247:F656–F664, 1984
7. Park CH, Maack T: Albumin absorption and catabolism by isolated perfused proximal convoluted tubules of rabbit. *J Clin Invest* 73:767–777, 1984
8. Beaufay H: Methods for isolation of lysosomes, in *Lysosomes in Biology and Pathology* (vol 2), Amsterdam, North-Holland Publishing Co., 1969, pp 515–546
9. Yamada H, Hayashi H, Natori Y: A simple procedure for the isolation of highly purified lysosomes from normal rat liver. *J Biochem* 95:1155–1160, 1984
10. Lehninger AL: Reversal of various types of mitochondrial swelling by adenosine triphosphate. *J Biol Chem* 234:2465–2470, 1959
11. Yamamoto K, Katsuda N, Himeno M, Kato K: Cathepsin D in rat spleen. *Eur J Biochem* 95:459–467, 1979
12. Milson DW, Rose FA, Dodgson KS: The specific assay of arylsulfatase C, a rat liver microsomal marker enzyme. *Biochem J* 128:331–336, 1972
13. Penington RJ: Biochemistry of dystrophic muscle. Mitochondrial

succinic-tetrazolium reductase and adenosine triphosphatase. *Biochem J* 80:649–654, 1961
14. De Duve C, Pressman BC, Gianetto R, Wattiaux R, Applmans F: Tissue fractionation studies 6. *Biochem J* 60:604–617, 1955
15. Baudhuin P, Beaufay H, Rahman LI, Sellinger OZ, Wattiaux R, Jacques P, De Duve C: Tissue fractionation studies 18. *Biochem J* 92:179–184, 1964
16. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC: Measurement of protein using bicinchoninic acid. *Anal Biochem* 150:76–85, 1985
17. Leighton F, Poole B, Beaufay H, Bandhuin P, Coffey JW, Fowler S, De Duve C: The large-scale separation of peroxisomes, mitochondria, and lysosomes from the liver of rats injected Triton WR-1339. *J Cell Biol* 37:482–513, 1968
18. Arborgh B, Ericsson JEL, Glaumann H: Method of the isolation of iron-loaded lysosomes from rat liver. *FEBS Lett* 32:190–194, 1973
19. Maunsbach AB: Isolation of kidney lysosomes, in *Methods in Enzymology, Biomembranes Part A* (vol 31), New York, Academic Press., 1974, pp 330–339
20. De Duve C, Wattiaux R: Function of lysosomes. *Ann Rev Biochem* 28:435–492, 1966
21. Geuze HJ, Slot JW, Strous GJAM, Hasilik A, Von Figura K: Possible pathways for lysosomal enzyme delivery. *J Cell Biol* 101:2253–2262, 1985
22. Geuze HJ, Slot JW, Schwaltz AL: Membranes of sorting organelles display lateral heterogeneity in receptor distribution. *J Cell Biol* 104:1715–1723, 1987
23. Buus S, Sette A, Grey HM: The interaction between protein-derived immunogenic peptides and Ia. *Immunol Rev* 98:115–141, 1987