BBA 65729

THE LYSOSOMAL LOCALIZATION OF SPHINGOLIPID HYDROLASES

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(Received December 15th, 1967)

SUMMARY

The distribution of three sphingolipid hydrolases was studied in subcellular fractions of rat liver and kidney tissue

Lysosomes contained the highest specific activity with regard to glucocerebrosidase, galactocerebrosidase, and sphingomyelinase.

Considerable sphingomyelinase activity is also found in mitochondria and microsomes which is probably not attributable to lysosomal contamination

INTRODUCTION

The catabolism of sphingolipids is catalyzed by a group of hydrolytic enzymes which bring about the sequential release of the various hexose or phosphorylamine components Studies on the subcellular distribution of a rat intestinal enzyme that cleaves the terminal galactose molecule from the ceramidetrihexoside, galactosylgalactosylglucosylceramide, revealed that most of the hydrolytic activity is contained in the particulate fraction which sediments between 700 and 12 000 $\times$ *g* (ref. 1) Ceramidase, β-galactosidase, and β-glucosidase from brain have been associated with the particles in the crude mitochondrial pellet which sediment between 15 000 and 20 000 $\times$ *g* (ref. 2) These enzymes have pH optima of 5 0. The glucocerebroside-cleaving enzyme from human spleen, and a glucosyl- and galactosylceramide-cleaving enzyme from rat intestinal tissue are most active in 800–9000 $\times$ *g* particulate fraction, and have pH optima of 6.0 (refs. 3, 4) Sphingomyelinase from rat liver[5] and from brain[2] is associated with particles sedimenting at 9000–20 000 $\times$ *g*, and has a pH optimum of 5 0. Maximal glucocerebrosidase activity in leukemic myelocytes correlates well with the subcellular fraction containing maximal acid phosphatase activity[6] These observations led to the postulation that the sphingolipid hydrolases might be predominantly lysosomal enzymes[6] Because lysosomes contain many hydrolytic enzymes having acid pH optima[7–9] including glucosidases, a galactosidase, and a phospholipase[10], the subcellular localization of glucocerebrosidase, galactocerebrosidase, and sphingomyelinase was critically examined in the present study

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METHODS AND MATERIALS

Preparation of lysosomes and other cellular fractions

Fractionation of liver and kidney tissue for preparation of lysosomes and other cellular fractions was performed as previously described[11,12]. The nuclear, mitochondrial, and microsomal fractions obtained in the course of the preparation of lysosomes were subjected to one or two washings Triton-filled lysosomes were prepared after the intraperitoneal injection of Triton WR-1339 according to the method of WATTIAUX[8].

Enzyme analyses

The enzymes which catalyze the hydrolysis of glucocerebroside, galactocerebroside, and sphingomyelin were assayed using ^{14}C-labeled substrates, as described in previous publications[3-5] For assay of glucocerebrosidase, 50-μl aliquots of suspended particles were used. 25-μl aliquots of particulate suspensions were used to assay for galactocerebrosidase and sphingomyelinase in order to remain within the proportional range of enzymatic activity Acid phosphatase was assayed with β-glycerophosphate as substrate as previously described[11]

Protein concentrations were determined by the method of Miller[13].

RESULTS

The subcellular distribution of glucocerebrosidase, galactocerebrosidase, and sphingomyelinase, in the order of their centrifugal fractionation, is shown in Tables I and II All three enzymes have their highest specific activity in lysosomes

The pattern of activities in the subcellular fractions from liver is shown in Fig. 1, in which the specific activity in lysosomes is taken as 100% This pattern is in accord

TABLE I

THE SUBCELLULAR DISTRIBUTION OF GLUCOCEREBROSIDASE, GALACTOCEREBROSIDASE AND SPHINGOMYELINASE IN RAT LIVER

Rat liver fraction	*Enzyme*			
	*Glucocerebrosidase**	*Galactocerebrosidase**	*Sphingomyelinase**	*Acid phosphatase***
Homogenate	6 12	4 03	11 9	35 5
Nuclear	2 07	1 57	2 99	20 6
Mitochondria	11 3	16 2	11 8	97 0
Lysosomes	49 2	62 5	25 8	615 0
Microsomes	9 24	3 56	14 6	40 0
Supernatant	4 96	4 46	12 5	10 5
Homogenate (Triton-treated rat)	3 94	3 98	6 91	26 4
Lysosomes (Triton-treated rat)	100 0	86 7	670 0	832 0

* Enzymatic activity is expressed as mμmoles of substrate hydrolyzed per mg of protein per h

** mμmoles/mg protein per min

TABLE II

THE SUBCELLULAR DISTRIBUTION OF GLUCOCEREBROSIDASE, GALACTOCEREBROSIDASE AND SPHINGOMYELINASE IN RAT KIDNEY

Rat kidney fraction	*Enzyme*			
	*Glucocerebrosidase**	*Galactocerebrosidase**	*Sphingomyelinase**	*Acid phosphatase***
Homogenate	7 77	10 0	9 78	48 0
Nuclear	10 7	15 1	14 2	52 6
Lysosomes	32 6	80 3	34 4	395.0
Mitochondria	7 77	4 84	12 0	39 4
Microsomes	2.92	1 18	2.76	28 8
Supernatant	4 86	5 33	6 09	15 5

* mμmoles of substrate hydrolyzed per mg of protein per h
** mμmoles/mg protein per min

with the distribution of protein and acid phosphatase activity found in studies[14] of twelve lysosome preparations The patterns for glucocerebrosidase and for galactocerebrosidase correspond closely to that for acid phosphatase, the most widely used marker enzyme for lysosomal activity. Furthermore, the distribution of glucocere-

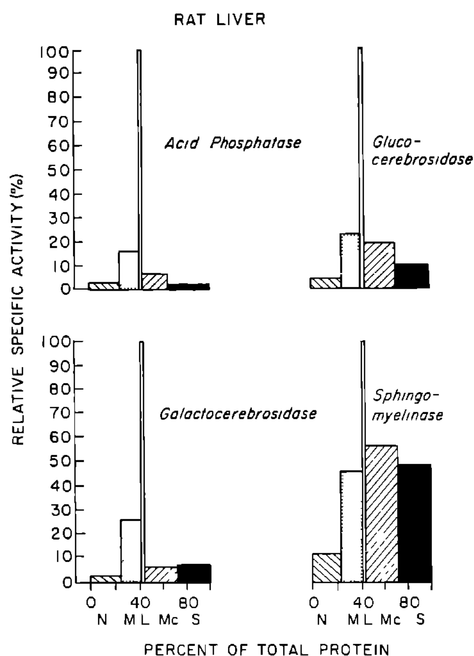

Fig. 1 Subcellular distribution of hydrolytic enzymes in liver The specific activity of the enzymes for each subcellular fraction is plotted on the ordinate as a percentage, relative to the specific activity in the lysosomal fraction The percentage of protein in each fraction of the homogenate is plotted on the abscissa· nuclear (N), mitochondrial (M), lysosomal (L), microsomal (Mc), and supernatant (S)

TABLE III

ENZYME BALANCE IN SUBCELLULAR FRACTIONS OF RAT LIVER ENZYMATIC ACTIVITY

Fraction	*Protein (mg/g liver)*	*Glucocerebrosidase**	*Galactocerebrosidase**	*Sphingomyelinase**	*Acid phosphatase***
Crude homogenate	210	1280	848	2500	7450
Nuclear	48	100	75	105	990
Mitochondrial	29	328	470	342	2820
Lysosomal	1	49	63	26	615
Microsomal	44	407	157	643	1760
Supernatant	64	311	285	802	670
Total in fractions	186	1195	1050	1918	6855
% of homogenate	89%	93%	124%	77%	92%

* Total substrate hydrolyzed per h in the respective fractions

** Total substrate hydrolyzed per min in the fractions

brosidase and galactocerebrosidase is similar to that found for various other lysosomal enzymes including arylamidase[12], sialidase[15], and phospholipase[10,16]

Enzyme recoveries were calculated as the sum of the activities of the fractions shown in Fig 1 divided by the total activity in the homogenate (Table III) The recoveries are: glucocerebrosidase, 93%, galactocerebrosidase, 124%; sphingomyelinase, 77%, acid phosphatase, 92%, and protein, 89%

In general, the fractions on either side of lysosomes—mitochondria and microsomes of liver, and nuclei and mitochondria of kidney, have only moderately high specific activities (Tables I and II) Previous studies, and a comparison with the acid phosphatase activity in these fractions, indicate that these activities in the fractions adjacent to lysosomes are due to contamination with lysosomes which are very heterogeneous The moderately high activity in the supernatant fractions could be related to enzymes released from fragmented lysosomes

The relatively high sphingomyelinase activity in mitochondrial, microsomal and supernatant preparations is in contrast with the low specific activity of acid phosphatase, glucocerebrosidase, and galactocerebrosidase in these fractions This pattern probably cannot be attributed solely to lysosomal contamination. It therefore seems likely that sphingomyelinase is not confined exclusively to lysosomes

DISCUSSION

The data obtained in the present experiments provide strong evidence for the subcellular localization of glucocerebrosidase, galactocerebrosidase, and a portion of sphingomyelinase in lysosomes Lysosomes fractionated from livers of untreated rats by the procedure used here[11], have been shown to be, at best, 86% lysomes, with about half the contamination from mitochondria and half from microsomes[17] Lysosomes prepared from rats treated with Triton WR-1339 have a median density of 1.1 g/ml, and density gradient centrifugation allows good separation from mitochondria and microbodies which have median densities in the 1 2 g/ml range In the first steps of fractionation, most of the microsomes are separated so that the final lysosome fraction is 90% or more lysosomes. Lysosomes fractionated from rat kidney have been

shown to be contaminated only with 8% mitochondria and a small amount of microsomes.

There is considerable evidence indicating a relationship between lysosomes and the metabolism of complex lipids Liver lysosomes, especially, are known to be involved in the digestion of mitochondria, endoplasmic reticulum, and other membrane structures containing complex lipids[18]. Following reactions catalyzed by their acid hydrolases, lysosomes may be converted into myelin body residue characterized by membrane arrays of lipid material[19,20]

Information on subcellular localization of sphingolipid hydrolases can conceivably add to the understanding of the pathophysiological alterations in sphingolipidoses. The metabolic defect in Gaucher's disease has recently been shown to be a deficiency of glucocerebrosidase[21] Electron microscopy of reticuloendothelial cells in patients with Gaucher's disease reveals the presence of inclusion bodies possessing a single membrane[22] The morphology of lysosomes, and the localization of glucocerebrosidase in lysosomes, suggest that the inclusions might be lysosomes congested with glucocerebroside. The frequently observed elevation in serum acid phosphatase in patients with Gaucher's disease may be due to the release of the lysosomal acid hydrolases from the glucocerebroside-loaded lysosomes during their transformation to the residual body form

Niemann–Pick disease is characterized by a deficiency of sphingomyelinase[23], and by the appearance of subcellular granules containing lamellar arrays of lipids Again, the electron microscopy of the granules and the subcellular localization of sphingomyelinase suggests that the granules are perhaps lysosomes loaded with unhydrolyzed sphingomyelin Furthermore, it is possible that Fabry's disease may also encompass a metabolic disorder involving lysosomes[1,24]

Foam cells morphologically rather similar to Gaucher cells have been found in the bone marrow of patients with chronic granulocytic leukemia, and increased levels of glucocerebrosidase activity have been measured in leukemic myelocytes[6] Lysosomal residual bodies and their associated phospholipids play a decisive role in the contribution of platelets to coagulation[25]. In this connection, the finding of foam cells in spleen and other tissues of patients with idiopathic thrombocytopenic purpura is of particular interest. Investigation of the activities of the sphingolipid hydrolases in a wide spectrum of seemingly unrelated clinical disorders may therefore be of importance in gaining more information about the emerging category of "lysosomal diseases".

ACKNOWLEDGEMENTS

Research at the University of California was supported by Public Health Service Research Grant AM 05609 from the National Institute of Arthritis and Metabolic Diseases. The authors gratefully acknowledge the assistance of Dr. S. MAHADEVAN and Miss C. DILLARD of the University of California

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