CHANGES IN LIPID COMPOSITION OF TRITOSOMES DURING LYSIS*

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Summary

Membrane phospholipids and free fatty acids were analyzed after lysis of lysosomes which had engulfed Triton WR 1339 (tritosomes). Significant elevations of lysophosphatidyl choline and lysophosphatidyl ethanolamine were seen. Phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin decreased significantly. Triglyceride also fell. The released free fatty acids paralleled closely the liberation of β-glucuronidase from the tritosomes. Thus, lipases hydrolyzed endogenous phospholipids and neutral lipids of tritosomes during incubation at 37°C at pH 5.0 to produce significant elevations of lysophospholipids and free fatty acids. The known surface-active properties of these latter compounds raise the possibility of their participation in the process of lysis of the tritosomes.

Introduction

The mechanism of lysis of lysosomal membranes is not known. Recent studies which localized the acid active phospholipases (1,2) and neutral lipid lipases

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(3,4) within the lysosome suggested the investigation of the changes in lipid content of this organelle during the process of lysis.

Methods

Triton WR 1339 (oxyethylated tertiary octylphenol polymethylene polymer) was injected intraperitoneally (1.0 mg/g body weight) into rats 4 days prior to isolation of hepatic tritosomes by discontinuous sucrose-gradient centrifugation (1). Livers were obtained after decapitation, homogenized at 4°C in 0.25 M sucrose, 0.005 M $MgCl_2$, 0.02 M Tris-H Cl, pH 7.2 and spun at 2000 xg max*. for 10 min. The pellet was re-homogenized in extraction buffer and spun again at 2000 xg max. The supernatants were combined and spun at 21,000 xg max. for 20 min. The pellet was resuspended in buffered sucrose and layered on top of a sucrose density gradient consisting of 6 ml of 1.32 M sucrose, 6 ml of 0.94 M sucrose & 9 ml of 0.82 M sucrose. After centrifugation for 2 hrs. at 86,000 xg max. in a SW 25.1 rotor, the material layering between the 0.25 M sucrose and 0.82 M sucrose was removed, diluted ten-fold with isotonic sucrose extraction buffer and spun at 69,000 xg max. for 30 min. The pellet was suspended to a protein concentration of 2-4 mg/ml in incubation buffer (0.25 M sucrose, 0.04 M Tris acetate, pH 5.0). Incubations at 37°C were performed in a Dubnoff shaking bath for up to one hour. Timed aliquots were taken for assay of lipids and β-glucuronidase (5). Lipids of incubated membranes were extracted (6) and protein determined (7). The chloroform phase of the lipid extracts was taken to dryness in a (Rotovapor) at 30°C and redissolved in 0.3 ml of chloroform:methanol (2:1). Phospholipids were separated on Supelco Redicoat plates in a two-dimensional system (8). Plates were sprayed with sulfuric acid, charred and spots removed for analysis of phosphorus (9). Neutral lipids were separated in a one-dimensional solvent system (10). Free fatty acids were isolated

*Maximal centrifugal force at the bottom of the centrifuge tube.

and esterified (11). Aliquots were analyzed on a Packard 7409 gas chromatograph equipped with a hydrogen flame ionization detector. Columns were packed with 10% EGSS-X on 100/120 mesh Gas Chrom P and run at temperatures of 180°C to 210°C. Data was analyzed using the Student t-test for paired results.

Results and Discussion

Table 1 lists the decreasing content of phospholipids of tritosomes at 0, 10, 20, 30 and 60 min. at 37°C, pH 5.0. The amount of choline and ethanolamine lysophospholipids progressively increased during the 60-min. incubation, while sphingomyelin, choline, ethanolamine, serine and inositol phospholipids decreased; bis phosphatidic acid remained relatively constant. The loss of recoverable phospholipid phosphorus per mg protein was

TABLE 1

Changes in Phospholipid Content of Incubated Tritosomes

Phospholipid	Control	10 min.	20 min.	30 min.	60 min.
LPC	1.8	2.0	2.7	5.3	11.6
PC	37.6	35.3	31.9	28.3	22.6
LPE	1.2	0.7	3.0	3.7	4.5
PE	12.2	12.5	10.9	9.4	6.9
BP	4.5	4.8	4.1	4.1	4.1
PS	3.3	3.4	2.8	2.7	1.6
PI	6.4	5.7	7.2	5.5	4.8
SM	10.0	7.7	6.4	4.5	2.1
Total	77.0	72.3	69.0	63.5	58.1

Phospholipid is reported as μ g atoms of P per 100 mg protein.

The data represents one experiment incubated up to one hour at 37°C, p H5.0 in Tris acetate buffer. LPC, lysophosphatidyl choline; P C, phosphatidyl choline; LPE lysophosphatidyl ethanolamine; PE, phosphatidyl ethanolamine; BP, bis-(monoacylglyceryl) phosphate; PS, phosphatidyl serine; PI, phosphatidyl inositol; SM, sphingomyelin.

significant and hydrolysis products not extractable in the chloroform phase probably accounted for the unrecovered phosphorus. In Table II the production of free fatty acids from the

Table II

Release of Free Fatty Acids During Lysosomal Lysis

Time	Free Fatty Acid	β-Glucuronidase
0	25	0
10 min.	80	24
20 min.	334	74
30 min.	381	85

Fatty acids are reported as nanomoles/mg protein after incubation at 37°C, pH 5.0. β-glucuronidase is represented as per cent enzymatic activity of the organelles after freezing and thawing ten times.

same experiment in Table I was greatest in the 10 to 20 min. period of incubation, and this time course paralleled that of 50 percent increase in β-glucuronidase activity in the supernatant. During a 30 min. incubation the triglyceride fraction was decreased by 65%, and contributed 98 nanomoles of FFA/mg protein/30 min., or about 25% of the released FFA. Table III presents the mean percentage change in the phospholipid phosphorus for five experiments. The most significant differences were seen in the choline and ethanolamine phospholipids and also in sphingomyelin. The increased percentages of lysophosphatidyl choline and ethanolamine and the lower percentage of sphingomyelin reflect the activity of phospholipase A and sphingomyelinase. Both lysosomal enzymes have optimal activity in the region of pH 5 (1, 12). The high degree of purity possible in tritosomes cannot be achieved in lysosomes that have not ingested Triton WR 1339 or a similar substance, and the characteristics of their membranes may be somewhat different from those of untreated lysosomes (13). Since only those lysosomes

Table III

Hydrolysis of Tritosome Lipids at Maximal Release of β-glucuronidase

Phospholipid	Control	Incubated
LPC	6.9 ± 2.0	19.2 ± 1.6*
PC	37.9 ± 2.8	28.0 ± 1.1*
LPE	3.6 ± 1.1	9.1 ± 0.3**
PE	13.3 ± 2.1	9.6 ± 1.3
BP	8.8 ± 1.7	11.9 ± 2.9
PS	4.3 ± 0.8	3.0 ± 1.0
PI	6.7 ± 0.6	6.8 ± 2.2
SM	16.9 ± 2.0	11.6 ± 3.3*

The means of five experiments are listed . Percent phospholipid phosphorus is reported. Abbreviations as in Table 1.

(*) $P < 0.05$; (**) $P < 0.01$.

capable of ingesting Triton WR 1339 are isolated, the results do not apply to nonphagocytic cells of the liver (1). However, it would be impossible to study lipid changes with any degree of specificity in native lysosomes due to the heavy contamination by mitochondria and other organelles which have their own characteristic structural lipids. The absence of cardiolipin in our tritosome preparations supports the view that contamination by mitochondria was limited. Furthermore, the enrichment of bis (monoacylglyceryl) phosphate in the tritosomes agrees closely with the 7% (4.1-11.0 range) reported for highly purified tritosomes (14).

The changes in free fatty acids and lysophospholipids described here indicate a large increase in the amount of surface-active lipids within the membrane at the time of lysis. This relationship may only be a temporal one that is not related to the mechanism of lysis of tritosomes. However, the possible contribution of these moieties to increased permeability

and ultimate lysis of the organelle is an intriguing question that remains to be investigated.

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