J. Membrane Biol. 34, 247–261 (1977)
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Membranous Localization and Properties of ATPase of Rat Liver Lysosomes

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Received 19 July 1976; revised 18 October 1976

Summary. Lysosomes isolated from rat liver were found to have ATPase activity (EC No. 3.6.1.3). Subfractionation of the lysosomes revealed a membranous localization of ATPase activity. The enzyme has half maximal activity at 0.2 mM ATP and is inhibited by high concentrations of ATP. The apparent K_m for divalent metal is 0.2 mM, and either Ca^{2+} or Mg^{2+} give maximal activity.

The ATPase activity has latency when lysosomes are isolated from rats treated with Triton WR-1339. This latency may be due to the presence of internalized sucrose because the activity of *L* fraction lysosomes is much less latent and Triton WR-1339 itself is not inhibitory. The latency of glucosaminidase, a marker enzyme for lysosomes, contrasts with the low latency of the ATPase and points to an ATPase with an exposed active site in intact lysosomes.

Intracellular protein degradation is important in higher animals; yet little is known of the mechanism of the degradative process, the enzymes and organelles involved and the regulation of the process [16, 39]. Lysosomes which contain hydrolytic enzymes presumably play a central role [6, 11, 31]. Simpson was the first to demonstrate that degradation is energy dependent [42] as it does not occur in liver slices incubated either anaerobically or in the presence of an uncoupler of oxidative phosphorylation.

If lysosomes are the site of degradation for intracellular proteins, then energy might be expended to either maintain an acidic internal pH or to transport proteins to be degraded. Mego and co-workers have suggested the existence of an ATP-driven proton pump in lysosomes which functions to maintain an acidic internal milieu [29]. This paper reports on the properties of a membranous ATPase from rat liver lysosomes.

Materials and Methods

Male albino rats weighing 150–300 g were obtained from either Charles River or Clinton Laboratories. Triton WR-1339 was purchased from Ruger Chemical Company. Other materials were from commercial suppliers, primarily Sigma.

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Preparations

(1) Differential centrifugation was performed as described previously [10]. The impure lysosomes of the *L*-fraction, compared to the homogenate, were 10× enriched in lysosomal enzymes.

(2) The flotation gradient technique [44] as modified by Leighton *et al.* [26] was used to prepare liver lysosomes from rats that had been treated with Triton WR-1339. In this technique the lysosomes present in a crude mitochondrial fraction (*ML*) are purified by flotation through a discontinuous sucrose gradient.

(3) Lysosomal membranes were isolated from lysosomes prepared from rats treated with Triton WR-1339 as described above. The lysosomes were stored overnight at −20°, thawed and diluted with an equal volume of cold 0.2 M NaCl, 50 mM Tris-SO_4, pH 8, 1 mM EDTA (STV). The mixture was centrifuged at 35,000 rpm for 40 min in a 50 Ti Spinco rotor ($g_{max}=110{,}000$). The turbid supernatant fractions were removed by complete decantation. The pellets were resuspended in STV with a glass-Teflon homogenizer, and centrifuged a second time in the same way. The final pellets contained about 20% of the protein and 60% of the ATPase activity of lysosomes and are designated lysosomal membranes.

(4) Hepatocyte plasma membrane has two opposite faces, the bile-canalicular face marked by 5′-nucleotidase and the blood-sinusoidal face marked by ouabain-sensitive Na^+, K^+-ATPase [13, 41, 46]. Plasma membranes enriched in 5′-nucleotidase were isolated from rat liver according to the procedure of Ray [37]. The purification was 27-fold compared to liver homogenate on basis of 5′-nucleotidase activity. Plasma membranes enriched in ouabain-sensitive ATPase were isolated from the microsomal fraction [10] by carrying out two sequential sucrose gradients. The microsomal fraction was layered on top of a 20–50% (w/w) linear sucrose gradient and centrifuged for 2 hr at 25,000 rpm in a Spinco SW 25.2 rotor ($g_{max}=107{,}000$). The fractions between densities 1.04 and 1.13 g/cc contained the ouabain-sensitive ATPase and were combined, mixed with an equal volume of STV, and pelleted by centrifuge at 35,000 rpm for 40 min in a Spinco 50 Ti rotor ($g_{max}=110{,}000$). The pellets were resuspended in 0.25 M sucrose and treated with digitonin as described [2]. The digitonin-membranes were layered on top of a 20–50% linear sucrose gradient and centrifuged as above. The fractions between densities 1.14 and 1.23 contained the ouabain-sensitive ATPase and were combined. This preparation has a specific ouabain-sensitive ATPase activity of 0.043 μmole/min/mg.

Enzyme Assays

(1) Cytochrome oxidase activity was determined spectrophotometrically in 20 mM potassium phosphate, pH 7.2, 0.1% Tween 80, 0.1 mM EDTA, and 1.5 mg/ml ferrocytochrome *c*. Ferrocytochrome *c* was prepared by dithionite reduction of a 30-mg/ml solution of Sigma type II-A cytochrome *c* and removal of excess dithionite by vortexing. The first order rate constant was obtained by the Guggenheim method [18].

(2) Glucosaminidase activity was measured using *p*-nitrophenyl-N-acetyl-β-D-glucosaminide as substrate [14]. The incubations were either for 30 min at 37° or as indicated in the text.

(3) ATPase activity was usually determined by measuring the enzyme catalyzed hydrolysis of ATP during a 10 min incubation at 37°. The reaction mixtures contained 0.1 M Tris-SO_4, pH 8, 1 mg/ml BSA, 2 mM $CaCl_2$, and 0.8 mM ATP in a 1.0-ml volume. The assay was initiated by adding ATP. During incubation, the assay mixtures in 13 × 100 mm glass tubes were continuously mixed by gentle reciprocal shaking. The reactions were stopped by the addition of 0.35 ml of 40% trichloroacetic acid to each tube and the mixture was clarified by centrifugation at 1800 rpm for 15 min in the Sorvall RC-3 centrifuge

(g_{max}=850). A 1.0-ml aliquot of each supernatant was analyzed for phosphate by the ammonium molybdate-ascorbic acid method [4]. When the concentration of $CaCl_2$ or ATP were varied in the determination of kinetic constants, time points were taken at 5, 10 and 15 min of incubation in order to monitor the linearity of the reaction. When latency of ATPase was measured $MgCl_2$ was substituted for $CaCl_2$. Also, 2 μg/ml oligomycin was added to completely inhibit mitochondrial ATPase. A continuous spectrophotometric assay of ATPase activity based on appearance of ADP was used to test whether lysosomal membranes contain an enzyme which acts on ATP to liberate ADP as well as phosphate. ADP was measured by disappearance of NADH with pyruvate kinase, lactate dehydrogenase and PEP. The reaction mixture contained sample, 4.3 mM PEP, 0.31 mM NADH, 6.9 mM $MgCl_2$, 570 mM KCl, 100 mM Tris-SO_4, pH 8.0, and 25 units of lactate dehydrogenase and pyruvate kinase in 1 ml. The reaction was initiated by addition of 0.8 mM ATP.

(4) 5′-Nucleotidase activity was measured by adding 2 mM Mg-AMP to a reaction mixture containing 100 mM Tris-SO_4, pH 8.0, 1 mg/ml BSA, and sample in a final volume of 1 ml. The mixture was incubated at 37° with gentle shaking for 60 min, and terminated by addition of trichloroacetic acid. Phosphate analysis was as described above for ATPase.

(5) Ouabain-sensitive, K^+, Na^+-ATPase activity was measured as described [15] except that 1 mM ouabain was employed.

A unit of enzyme activity is the amount which produced 1 μmole of product per min.

Latency Measurements

Enzymes located inside organelles are inactive, if their substrates are impermeant to the organelle membranes, unless the membranes are ruptured. This property is called latency [8] and is normally an indicator of membrane integrity. Measurements were carried out on intact lysosomes prepared the same day. Maximal activity (0% latency) was achieved by disrupting the preparation with a Branson Sonifier Model S 125 equipped with a microtip probe; 2 ml of sample was sonicated at 0° for 15 sec at power setting 4. Although Triton X-100 is usually used to demonstrate maximal activity, it was not used in this study because of its interference in the measurement of phosphate [12]. Enzyme assays were performed as described above except that 0.25 M sucrose was added to all reaction mixtures, and all incubation times were 10 min. The fractional amount of latent activity was calculated by subtracting the activity of intact lysosomes from that of sonicated lysosomes and dividing this difference by the activity of sonicated lysosomes.

Protein was measured as described by Lowry [27] except that 0.4% sodium deoxycholate was used to clarify membranous preparations.

Results

ATPase Activity Associated with Lysosomes

When the lysosome-enriched fraction (*L*) was prepared from untreated rat liver by differential centrifugation, the distribution of ATPase in the fractions was different from that of cytochrome oxidase (mitochondria) and glucosaminidase (lysosomes), as shown in Fig. 1. Since a number of known enzymes possess ATPase activity the low relative activity of ATPase in *L* certainly did not rule out existence of an ATPase

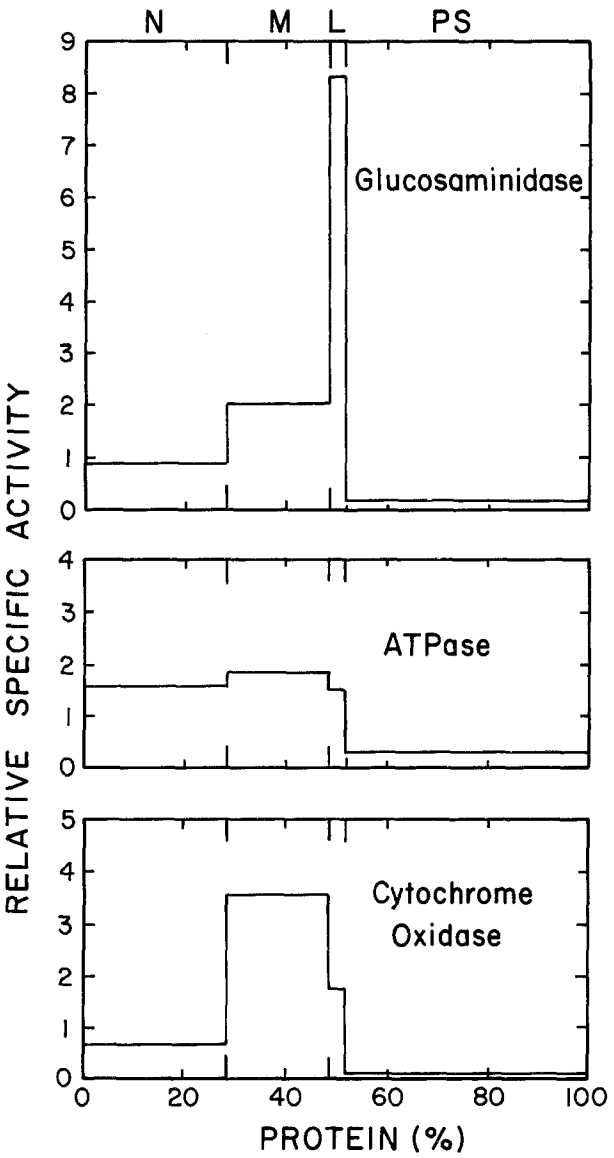

Fig. 1. Differential centrifugation pattern of ATPase compared with glucosaminidase and cytochrome oxidase. Nuclear (*N*), mitochondrial (*M*), light mitochondrial (*L*), and post-mitochondrial (*PS*) fractions were prepared by centrifugation as described in Materials and Methods. The results are plotted in histogram form as suggested by de Duve [10] so that the area of each bar represents the portion of the enzyme activity in that fraction and the relative activity represents the enrichment of the enzyme activity in that fraction. Recoveries were 99, 100, 116, and 104% of the glucosaminidase, ATPase, cytochrome oxidase and protein, respectively; amounts per g liver were 5.82 U, 37.4 U, 1943 min^{-1}, and 186 mg

unique to lysosomes, especially since lysosomes constitute but a small fraction (<2%) of liver protein. In order to test for the presence of a lysosomal ATPase, additional purification was necessary.

Lysosomal preparations isolated by the flotation method have been reported to be 80% pure and 50-fold enriched in the activity of specific marker enzymes [26]. Glucosaminidase measurements confirmed that we were able to obtain at least this level of purity (Table 1). These preparations have a high specific activity for ATPase. When livers from untreated rats are carried through the purification procedure, neither ATPase nor glucosaminidase activity was found in the region of the sucrose gradient where lysosomes normally accumulate. Since Triton WR-1339 affects only the density of lysosomes [21], it is very unlikely that the ATPase activity is from a contaminant.

The ATPase activity of lysosomes is not sensitive to oligomycin, Table 2. This insensitivity is contrasted with that of the F_1 (ATPase) of mitochondria. High concentrations of oligomycin are known to cause nonspecific inhibition of ATPases [5]; consequently the progressive inhibition of the lysosomal ATPase by concentrations of oligomycin greater than 5 μg/ml is not unexpected. The ATPase activity of lysosomes cannot be attributed to mitochondrial contamination; however, the lysosomal

Table 1. Purification of lysosomes from liver after treatment of rats with Triton WR-1339[a]

Fraction	ATPase		Glucosaminidase		Protein
	(unit/g liver)	(unit/mg protein)	(unit/g liver)	(unit/mg protein)	(mg/g liver)
E+N (Total liver)	13.00	0.0755	3.38	0.0196	172.1
ML	5.87	0.1668	1.80	0.0511	35.2
Gradient:					
1	0.006	0.0594	0.002	0.0198	0.101
2 (Lysosomes)	0.119	0.1604	1.00	1.348	0.742
3	0.054	0.0874	0.14	0.2265	0.618
4	5.017	0.1733	0.38	0.0131	28.94

[a] The mitochondrial fraction obtained by differential centrifugation (*ML*) was resuspended in 45% sucrose (w/w) and layered on the bottom of a discontinuous sucrose gradient as described previously [26]. After centrifugation, four successive fractions were collected from the top. Enzyme and protein assays were performed as in Materials and Methods. Recoveries were 88, 84 and 86% for ATPase, glucosaminidase and protein, respectively. On a relative specific activity basis [*cf.* 10], the glucosaminidase of lysosomes was 69× that of total liver; on a specific activity basis, 69×.

Table 2. Oligomycin inhibition of the ATPase activities of rat liver mitochondria and lysosomes[a]

Addition	ATPase Activity			
	Mitochondria		Lysosomes	
	(μmole/min/mg)	(%)	(μmole/min/mg)	(%)
None	0.146	100	0.726	100
Oligomycin 0.5 μg/ml	0.013	8	0.760	105
Oligomycin 1.5 μg/ml	0.006	4	0.729	100
Oligomycin 5.0 μg/ml	0.004	3	0.574	79

[a] An *M* fraction obtained by differential centrifugation as described [10] was used as mitochondria. The membranous fraction of lysosomes prepared using Triton WR-1339 as described in Materials and Methods was used as the source of lysosomal ATPase. When mitochondria are subfractionated in this manner no ATPase activity remains membranous. Oligomycin was added from a 100 μg/ml stock solution in 95% ethanol.

ATPase is but 3% of the total (Table 1) and, therefore, the possibility of contamination will be further explored.

In the first place, if one disregards mitochondrial ATPase activity, then the lysosomal activity accounts for more than 10% of the total. In view of the specific effect of Triton WR-1339 [21], exogenous contamination is slight. However plasma membrane is a reasonable source of endogenous contamination because endocytosis would cause plasma membrane to become part of the lysosomal membrane and Triton WR-1339 is known to promote endocytosis [43]. Accordingly plasma membranes were prepared, and their Ca^{2+}-ATPase activities were compared to that of lysosomes (Table 3). Plasma membrane with 5′-nucleotidase activity is as active in ATPase as lysosomal membranes, whereas plasma membrane with ouabain-sensitive ATPase activity is much less active. Since endocytosis of Triton WR-1339 would involve the blood-sinusoidal face of the plasma membrane which is marked by ouabain-sensitive ATPase [13, 41, 46] and since this membrane has little Ca^{2+}-ATPase activity, the data strongly suggest that the ATPase activity of lysosomes is not due to contamination resulting from endocytosis. The Ca^{2+}-ATPase of plasma membrane marked by 5′-nucleotidase requires comment. This membrane is from the bile-canalicular face of the plasma membrane [13, 41, 46]. If anything the Ca^{2+}-ATPase in these membranes may be derived from lysosomes during exocytosis. Although, in view of the occurrence of Ca^{2+}-ATPase in many membranes active in endo- or exocy-

Table 3. Comparison of lysosomal and plasma membrane Ca^{2+}-ATPase activities[a]

Preparation	Ca^{2+}-ATPase Activity (μmole/min/mg)
Lysosomal membrane	0.61
Plasma membrane	
with ouabain-sensitive ATPase	0.03
with 5′-nucleotidase	0.77

[a] Lysosomal membranes were prepared from lysosomes as described in Materials and Methods. Plasma membrane with 5′-nucleotidase activity was prepared from the low speed pellet of rat liver homogenate as described [37]. Plasma membrane with ouabain-sensitive ATPase activity was obtained from microsomes [10] by digitonin treatment and sucrose gradient centrifugation as described in Materials and Methods. Ca^{2+} ATPase activity was measured with 2 mM $CaCl_2$ and 0.8 mM ATP at pH 8 as described in Materials and Methods under Enzyme Assays, ATPase.

tosis [7, 33, 34, 38, 47, 48], the bile-canalicular face of the plasma membrane may also have need of a Ca^{2+}-ATPase.

Properties of the ATPase Associated with Lysosomal Membranes

Subfractionation of lysosomes was carried out to test whether ATPase activity is associated with the membrane. As shown in Fig. 2, the majority of ATPase activity is membranous either by appearance of phosphate [*cf.* 40] or by appearance of ADP in the continuous, coupled assay. The fractionation conditions were not favorable for nonspecific absorption of soluble protein to membrane as glucosaminidase remained soluble. Since this simple subfractionation step separates the particulate ATPase from soluble lysosomal proteins, a 3-fold purification is obtained. The nature of the 20–40% of the ATPase which appears to be soluble could be due in part to nonspecific, soluble acid phosphatase(s) [10, 20].

ATPase Kinetic Properties

The specific ATPase activity reported in these experiments is much greater than previously reported [24]. Since a much higher concentration of ATP had been used, the dependence on ATP concentration was considered. Fig. 3 shows the saturation curve for the ATPase activity of lysoso-

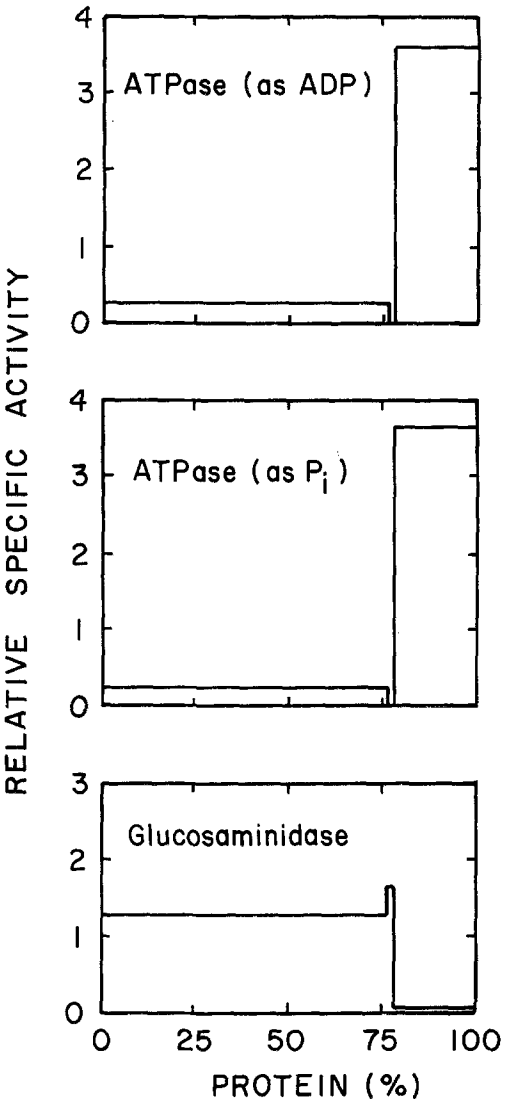

Fig. 2. Subfractionation of lysosomes into soluble and membranous fractions by differential centrifugation. Lysosomes were prepared as discussed in Materials and Methods. After freezing and thawing of the lysosomes, subfractionation was carried out by diluting with an equal volume of cold 0.2 M NaCl, 50 mM Tris-SO_4, pH 8, and 1 mM EDTA (STV) and centrifuging at 110,000 *g* as described in Materials and Methods. The supernatant fraction was decanted, and the pellet was resuspended in STV and recentrifuged. Enzyme and protein assays were performed as described in Materials and Methods. The first and second supernatant fractions contained 75 and 2% of the protein, respectively; the final pellet contained 23% of the protein and is designated lysosomal membrane. The results are plotted as in Fig. 1 except that the first and second bars represent respective supernatant fractions, and the last one represents the membranous fraction

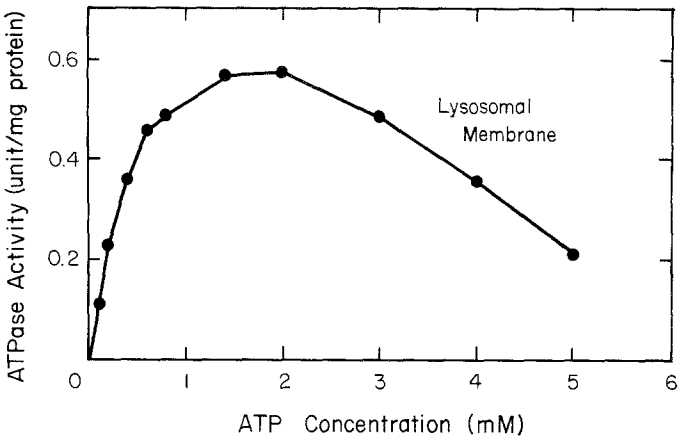

Fig. 3. Effect of ATP concentration on ATPase activity of lysosomal membranes. The membranous fraction was obtained from lysosomes isolated by the Triton technique as described (*see* Materials and Methods). Assays were with 20 mM $CaCl_2$ and the indicated amount of ATP. Phosphate production was measured at 5, 10 and 15 min

mal membranes. High levels of ATP were inhibitory. This inhibition was probably not due to ATP complexing all of the free calcium because the total calcium concentration was 20 mM while ATP was at most 5 mM.

In view of the marked inhibitory effects of high concentrations of ATP, the possibility of similar effects at high metal ion concentrations was also considered. Ca^{2+} and Mg^{2+} ATPase activities were measured. Fig. 4 shows metal ion saturation curves for the ATPase activity. The lysosomal ATPase is equally active with Ca^{2+} or Mg^{2+} and is not inhibited by high concentrations of metal.

Additional kinetic studies were carried out on the membranous ATPase of lysosomes with other substrates and possible inhibitors. The enzyme hydrolyses GTP nearly as well as ATP (Table 4). Although enzyme activity is less with ADP and AMP than with ATP, the activity with ADP may be sufficient to account for the previously described nucleoside diphosphatase (ADPase) activity of lysosomes [45]. Tartrate is an inhibitor of acid phosphatase [24] and as reported for the ADPase activity, it is not an effective inhibitor of the ATPase. EDTA and EGTA are potent inhibitors, whereas concanavalin A and DTT are somewhat stimulatory.

Various metal ions were tested and Ca^{2+} and Mg^{2+} were the best activators of the lysosomal ATPase (Table 5). A mixture of 0.2 mM each Ca^{2+} and Mg^{2+} did not give additive rates expected of two different enzymes but instead gave a rate close to the 2/3 V_{max} value of 3.30

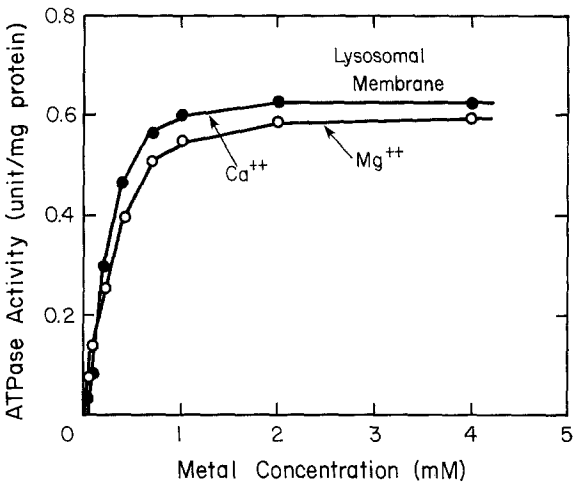

Fig. 4. Effect of calcium and magnesium concentrations on ATPase activity of lysosomal membrane. Lysosomal membranes were obtained as described in Fig. 3. Assays were in the presence of 50 μM each EDTA and EGTA and were initiated by adding 0.8 mM ATP. In the absence of chelating agents ATPase activity does not show a strict dependency on added divalent metals. Phosphate production was measured at 5, 10 and 15 min as discussed in Materials and Methods. The data points shown are with Ca^{2+} (solid circles) and with Mg^{2+} (open circles)

expected of a single enzyme activated by either Ca^{2+} or Mg^{2+}. Mn^{2+} is about half as effective as Ca^{2+} or Mg^{2+}. Na^{+} and K^{+} are not activators.

The kinetic results are consistent with the ATPase being a single enzyme in the lysosomal membrane. The next consideration is whether the ATPase is suitably located in the lysosomal membrane so as to utilize cytoplasmic ATP.

ATPase Latency. If lysosomes utilize cytoplasmic ATP, then the active site of the ATPase must be exposed and the activity of intact lysosomes should not be latent. However, lysosomes isolated from rats treated with Triton WR-1339 do display latent ATPase activity typical of soluble lysosomal enzymes (Table 6). Inasmuch as lysosomes prepared by the Triton technique may be functionally altered [17], the latency of ATPase activity was examined in the unaltered, less pure *L* fraction obtained by differential centrifugation [10]. Table 6 shows that *L* fraction ATPase is much less latent than *L* fraction glucosaminidase. The presence of oligomycin during these measurements precludes the possibility of mitochondrial ATPase activity.

Table 4. Effect of various substrates and other compounds on lysosomal ATPase[a]

Addition(s)	Activity
0.2 mM ATP	100%
0.2 mM GTP	91
0.2 mM TTP	67
0.2 mM CTP	28
0.2 mM ADP	39
0.2 mM AMP	7
0.8 mM ATP	100
0.8 mM GTP	100
0.8 mM TTP	84
0.8 mM CTP	41
0.8 mM ADP	43
0.8 mM AMP	17
0.8 mM ATP	100
0.8 mM ATP plus concanavalin A	114
0.8 mM ATP plus 5 mM DTT	112
0.8 mM ATP plus 20 mM tartrate	64
0.8 mM ATP plus 2 mM EDTA	0
0.8 mM ATP plus 2 mM EGTA	3
0.8 mM ATP plus 0.2 mM acetazolamide	103

[a] The membranous fraction of lysosomes obtained with Triton WR-1339 was prepared as in Materials and Methods and was used as enzyme. Reaction mixtures contained 100 mM Tris-SO_4, pH 8.0, 2 mM $CaCl_2$, 1 mg/ml BSA, enzyme, and the materials listed under "Additions". After 10 min incubation at 37°, phosphate released was analyzed as in Materials and Methods

Table 5. Effect of various metal ions on lysosomal ATPase activity[a]

Addition	ATPase Activity (mU)
0.2 mM $CaCl_2$	2.03
0.2 $MgCl_2$	2.37
0.2 each $CaCl_2$ and $MgCl_2$	3.37
0.2 $MnCl_2$	1.51
2.0 mM $CaCl_2$	5.44
2.0 $MgCl_2$	4.41
2.0 $MnCl_2$	2.19
2.0 mM $MgCl_2$ plus 100 mM KCl	3.84
2.0 mM $MgCl_2$ plus 15 mM KCl and 145 mM NaCl	3.65

[a] The membranous fraction of lysosomes was obtained as in Materials and Methods. Reaction mixtures contained 0.6 mM ATP, 100 mM Tris-SO_4, pH 8, 1 mg/ml BSA, 50 μM each EDTA and EGTA, and metal ion as indicated and were incubated for 10 min. Phosphate released was measured as in Materials and Methods.

Table 6. Effect of sucrose on the ATPase activity of *L* Fraction[a]

Addition (mM)	ATPase activity (unit/mg)
sucrose, 0	0.050
sucrose, 250	0.026
sucrose, 500	0.009

[a] The *L* fraction was prepared by differential centrifugation and ATPase assayed as described in Materials and Methods except that 2 μg/ml oligomycin was added to inhibit mitochondrial ATPase activity.

Table 7. The latent properties of lysosomal enzymes[a]

Preparation	ATPase		Glucosaminidase	
	(unit/mg)	(latency)	(unit/mg)	(latency)
Intact Tritosomes	0.13	65%	0.72	64%
Sonicated Tritosomes	0.38		2.01	
Intact *L* Fraction	0.014	35%	0.065	81%
Sonicated *L* Fraction	0.022		0.350	

[a] Lysosomes were prepared by either differential centrifugation (*L* fraction) or the flotation technique after treatment of the rats with Triton WR-1339 (Tritosomes) as described in Materials and Methods. ATPase and glucosaminidase were measured as described above under Latency Measurements.

In consideration of the differences in latency between Tritosomal and *L* fraction ATPase, the effect of Triton WR-1339 and sucrose was tested. Triton WR-1339 did not inhibit ATPase activity even at 2%. On the other hand, sucrose was quite inhibitory (Table 7). In view of the fact that the flotation procedure for isolation of Tritosomes involves a resuspension in 1.6 M sucrose, our latency results may very well be due to a slight uptake of sucrose by Tritosomes.

Discussion

An important question this study fails to answer is the extent of damage caused by endocytosis of Triton WR-1339. Two points are pertinent: (1) the inhibition of ATPase is not due to Triton directly and may in fact be due to sucrose which penetrates during isolation and

(2) the diminished latency of *L* fraction ATPase compared to glucosaminidase means that the active site of the ATPase is exposed to the cytoplasm.

The presence of a membranous ATPase in liver lysosomes poses several interesting possibilities, some of which pertain to the bioenergetics of protein catabolism. In the first place a membranous enzyme activated by Ca^{2+} may regulate entry of material into the lysosome. In view of the widely observed stimulation of fusion by Ca^{2+} [7, 25, 28, 30, 32, 33, 34, 38, 47, 48] one can consider the possibility that the ATPase regulates the free Ca^{2+} concentration, e.g., by pumping Ca^{2+} into the lysosome. Thus the ATPase may function in the membrane fusion process by means of which material from endocytotic vesicles and autophagic vacuoles is taken into lysosomes for digestion. This possibility is consistent with recent hypotheses on membrane fusion [1, 35] and should be further investigated. The same ATPase may function in all membranes active in fusion, e.g., the granules of chromaffin cells [3, 19], leukocytes [47, 48], platelets [38] and salivary glands [7]. Fusion may depend on its presence in both the plasma and granule membranes. Alternatively the enzyme may be important in pumping Ca^{2+} out of lysosomes as has been suggested for a similar Ca^{2+}-ATPase in erythrocytes [23].

Secondly, lysosomes are known to have an acidic interior which probably requires energy [9]. By analogy with mitochondrial F_1 (ATPase) [22], especially as found in submitochondrial particles [36], the presence of a membranous ATPase offers a very attractive candidate for a proton pump. As was the case for F_1 (ATPase) [22], definitive experiments will depend on purification of the ATPase and reconstitution of an active pump.

E. Cornell and F. Di Mario provided skillful technical assistance for which the author is grateful. The author benefited from discussions with E.W. Westhead, R.C. Fuller and T.L. Mason. The patience and typing of L. Havis and J. Fournier are appreciated.

This work was supported by Grant GM 21260 from the National Institute of General Medical Science of the National Institutes of Health.

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