NUCLEOSIDE DIPHOSPHATASE IN PURIFIED PREPARATIONS OF RAT LIVER LYSOSOMES

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1. Introduction

When performing their heterophagic function, the lysosomes and the endocytosis vacuoles have to fuse in order to allow contact between the acid hydrolases and the endocytosed compounds [1]. Such a process suggests some structural similarity between the lysosome membrane and the plasma membrane from which the endocytosis vesicle membrane is made. Several enzymes are known to be linked to the plasma membrane; thus one possible way of comparing the plasma membrane with the lysosome membrane is to study their enzymic content. Recently we have shown that a nucleoside diphosphatase was associated with the liver plasma membrane [2]. The present work indicates that a similar enzyme is present in purified lysosome preparations and is found mainly in the membranous fraction of these preparations.

2. Experimental

Experiments were performed with male and female Wistar rats weighing 200–300 g. Density-gradient experiments were performed using a method similar to that of Beaufay et al. [3]. Plasma membranes were isolated according to the method of Neville [4] with the modifications introduced by Emmelot et al. [5]. Purified preparations of lysosomes were made according to Trouet [6]. Cytochrome oxidase, acid phosphatase and glucose-6-phosphatase activities were measured according to the method of de Duve et al. [7], NADH-cytochrome *c* reductase according to Ernster et al. [8], ADPase by the method of Wattiaux-De Coninck and Wattiaux [2], protein by the method of Lowry et al. [9]. Units of enzymatic activity are defined as the amount of enzyme causing the decomposition of 1 μmole of substrate per min under the conditions of the assay. One cytochrome oxidase unit is defined as the amount of enzyme causing a logarithmic decrease of the concentration of reduced cytochrome *c* by 1 unit per min per 100 ml of incubation mixture [7].

3. Results

3.1. *ADPase distribution after isopycnic centrifugation of a rat liver mitochondrial fraction*

Rat liver lysosomes sediment mainly with the mitochondrial fraction (*ML* according to de Duve et al. [7]) after differential centrifugation in isotonic sucrose. When this fraction is submitted to isopycnic centrifugation in a sucrose gradient, lysosomes exhibit a relatively flattened distribution curve as indicated in fig. 1 by the acid phosphatase distribution. The distribution of mitochondria is more homogeneous as ascertained by the cytochrome oxidase pattern. A significant proportion of the ADPase activity of the homogenate is recovered in the mitochondrial fraction [2]. Distribution of the enzyme after isopycnic centrifugation is illustrated in fig. 1. Fig. 1 also illustrates the distributions observed when the mitochondrial fraction is isolated from the liver of a rat injected with the non-ionic detergent Triton WR-1339. As shown by Wattiaux et al. [10], the density of rat liver lysosomes is strongly decreased by the Triton treatment and acid hydrolases are found well sepa-

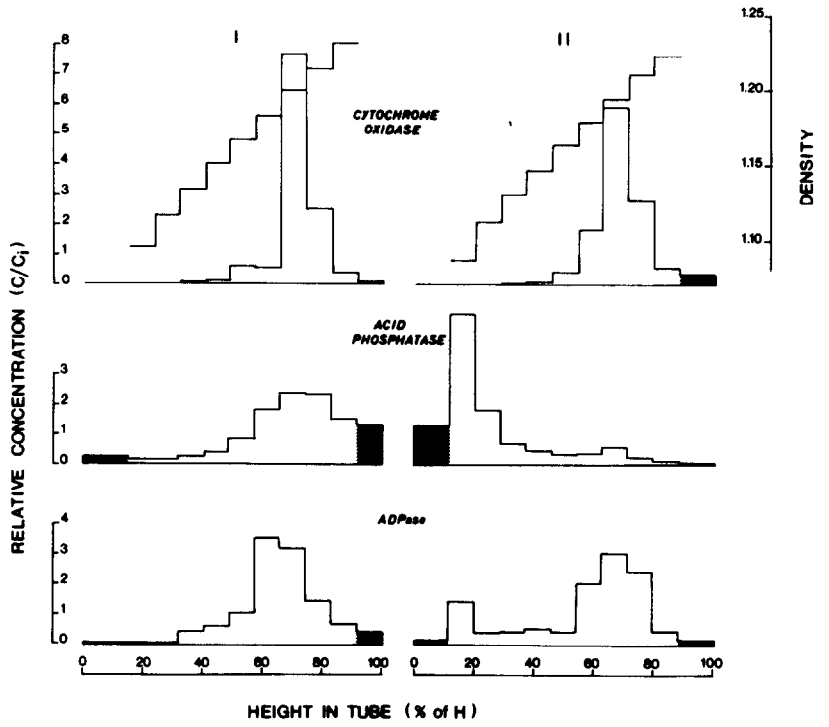

Fig. 1. Density equilibration of particle-bound enzymes after centrifugation (for 2.5 hr at 39,000 rpm in head SW39 of the Spinco model L-HV preparative ultracentrifuge) of a mitochondrial fraction of rat liver through a 0.776 to 3.419 molal sucrose gradient in water. The particles were initially layered on the top of the gradient. Abscissa: percentage of the height of the liquid column in tube (H); ordinate: relative concentration i.e. ratio of the observed activity (c) to that which would have been found if the enzyme had been homogeneously distributed throughout the whole gradient (c_i). Shaded blocks (▨) are used for the top and bottom subfractions to indicate that they include material falling beyond the limits of the gradient. (I) preparation from a control animal, (II) preparation from an animal given one intravenous injection of Triton WR-1339 (170 mg in 1 ml isotonic saline); the animal was killed four days after the injection. Recoveries in gradients were 70.8 and 95.0% for cytochrome oxidase, 103.0 and 114.1% for acid phosphatase, 100.9 and 100.2% for ADPase.

rated from cytochrome oxidase in the regions of low density of the gradient. In this case, ADPase found in the mitochondrial fraction exhibits a bimodal distribution: the greater part of the activity (about 80%) has an unchanged distribution; the other part has migrated like acid phosphatase and a significant peak appears at the same position as the hydrolase peak.

3.2. *ADPase activity of purified lysosomal preparations*

These results suggest that an ADPase activity is associated with the lysosomes. Indeed, the purified preparations of the granules obtained by the method of Trouet [6] possess such an activity. If the granules are ruptured and the lysosome preparation (*T*) is divided by centrifugation into a membrane fraction (*Tm*) and a soluble fraction (*Ts*), most of the ADPase activity is recovered in the membrane fraction (table 1). Results recorded in table 2 allow a comparison between the activity found in the homogenate, the purified lysosome preparation and in the plasma membrane preparation isolated from the same liver. Several reference enzymes have also been determined in these experiments. On a protein basis, the acid phosphatase activity of the *T* preparation is 50 to 60 times higher than that of the homogenate. The contamination by mitochondria and endoplasmic reticulum membranes is minimal as indicated by the low activity of cytochrome oxidase, glucose-6-phosphatase and NADH-

Table 1

ADPase activity of purified lysosomal preparations. An aliquot of the lysosome preparation *T* was sonically treated at 0°C for three 1-min periods with the fine tip of a MSE sonifier (Measuring and Scientific Equipment, London, England) set at position 7 on amplitude indicator: then the preparation was divided by centrifugation at 3.7×10^6 *g* min in a membrane fraction *Tm* and a soluble fraction *Ts*. (a) units/g fresh weight of liver; (b) units/mg of protein.

Experiment	*T*		*Tm*		*Ts*	
	(a)	(b)	(a)	(b)	(a)	(b)
1	0.58	0.51	0.56	1.25	0.07	0.09
2	0.52	0.35	0.45	0.84	0.05	0.05
3	0.58	0.35	0.50	0.98	0.06	0.07
4	0.69	0.50	0.68	1.24	0.08	0.09
5	0.60	0.61	0.55	1.60	0.09	0.13

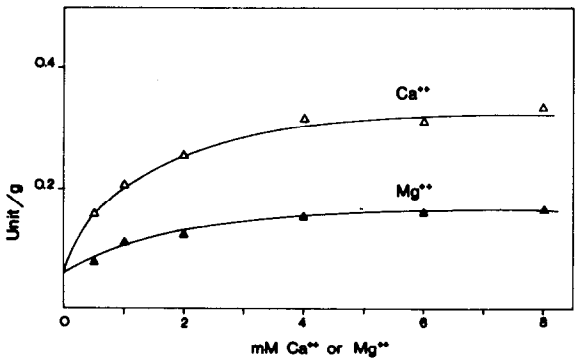

Fig. 2. Effect of Ca^{2+} and Mg^{2+} on the ADPase activity of the membrane fraction of a purified lysosome preparation.

Table 2

Enzymic activities of homogenate, purified lysosome and plasma membrane preparation. (a) units/g fresh weight of liver; (b) units/mg protein. For explanation of *T*, *Tm* and *Ts* see table 1; *Mb*: plasma membrane preparation.

Exp.	Enzymes	Homogenate		*T*		*Tm*		*Ts*		*Mb*	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
1	Acid phosphatase	9.8	0.043	2.73	2.18	1.98	3.9	0.89	1.22	0.012	0.013
	Cytochrome oxidase	23.9	0.105	0.002	0.0016	0.002	0.004	0	0	0.003	0.003
	Glucose-6-phosphatase	21.0	0.091	0.09	0.071	0.10	0.20	0.07	0.09	0.024	0.027
	NADH-cyt. *c* reductase	17.0	0.075	0	0	0	0	0	0	0.04	0.044
	ADPase	9.8	0.043	0.60	0.48	0.49	0.96	0.08	0.11	0.78	0.86
	Proteins (mg/g fresh weight)	218.0	-	1.25	-	0.51	-	0.73	-	0.90	-
2	Acid phosphatase	8.1	0.035	1.86	2.09	0.94	3.04	1.0	1.49	0.05	0.06
	Cytochrome oxidase	30.5	0.133	0.006	0.007	0.006	0.019	0	0	0.004	0.005
	Glucose-6-phosphatase	15.2	0.066	0.12	0.13	0.08	0.26	0.06	0.09	0.03	0.036
	NADH-cy. *c* reductase	20.0	0.087	0.01	0.011	0.008	0.026	0	0	0.05	0.06
	ADPase	9.0	0.040	0.44	0.48	0.31	1.0	0.12	0.18	1.13	1.37
	Proteins (mg/g fresh weight)	228.2	-	0.89	-	0.31	-	0.67	-	0.82	-

cytochrome *c* reductase; thus the *T* fraction corresponds to a highly purified lysosomal preparation. Indeed, the specific activity of ADPase is more than 10 times higher than that of the homogenate; in the membrane fraction (*Tm*) where most of the enzyme is recovered, a 25-fold purification is observed. On the other hand, the activity of the reference enzymes in the plasma membrane preparation indicates that it is only slightly contaminated by lysosomes, mitochondria and endoplasmic reticulum membranes. The specific activity of ADPase is comparable to that found in the lysosome membrane fraction (*Tm*).

Some properties of the ADPase present in the purified lysosome preparations are similar to those of the enzyme associated with the plasma membrane [2]. As illustrated by fig. 2, Mg^{2+} and Ca^{2+} stimulate

the reaction but its rate in the presence of Ca^{2+} is higher than that observed in the presence of Mg^{2+}. The optimum pH of the reaction is between 6.5 and 7.0 (fig. 3). Deoxycholate does not increase the activity. At equal concentration (5 mM) ADP, UDP and GDP are hydrolyzed at a comparable rate and CDP at a lower rate. The activity is not inhibited by tartrate.

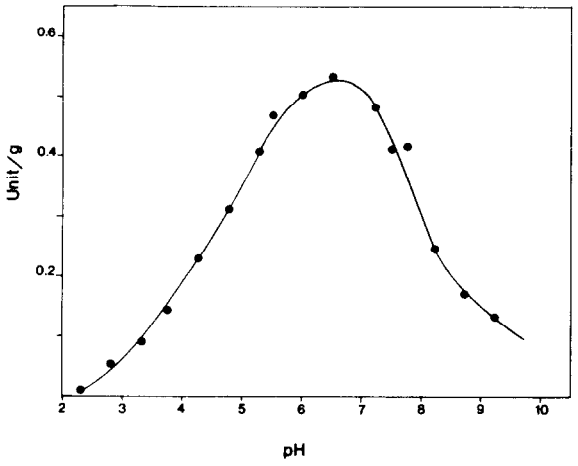

Fig. 3. Effect of pH on ADPase activity of the membrane fraction (*Tm*) of a purified lysosomal preparation. The tests were performed in presence of 5 mM $CaCl_2$; the reaction mixtures contained 50 mM buffer. The buffers used were: glycine-HCl from pH 2.3 to 3.3; acetic acid-sodium acetate from pH 3.75 to 5.8; sodium cacodylate-HCl from pH 6.0 to 7.5; tris buffer from pH 7.5 to 9.2.

4. Discussion

As shown by Wattiaux et al. [10], Triton WR-1339 is endocytosed by the liver and is accumulated in the lysosomes with water; this is why the density of the granules decreases considerably after an injection of the detergent. The phenomenon is specific for the lysosomes, other organelles present in the mitochondrial fraction like mitochondria and peroxisomes are not affected by the treatment. Therefore, the effect of Triton WR-1339 injection on ADPase distribution in a sucrose gradient suggests that an ADPase activity is associated with the lysosomes. The experiments performed on purified lysosomal preparations indicate that the ADPase is mostly associated with the membrane fraction where it exhibits a similar activity, on a protein basis, to that found in purified plasma membranes. Therefore it would be difficult to explain the ADPase activity only by a contamination of the lysosomal preparation with plasma membrane fragments. We would have to suppose that these fragments are endowed with a much higher ADPase activity on a protein basis than those isolated by the method of Emmelot et al. [5] or that the lysosomal membrane proteins represent only a small proportion of the total proteins of the lysosomal preparation. Moreover, the density of these fragments would have to be affected in a similar way to the lysosomes by the Triton WR-1339 treatment of the animal.

According to Brightwell and Tappel [11], purified acid phosphatase is able to hydrolyze ADP. It is unlikely that the ADPase activity of the purified lysosomal preparation is due to acid phosphatase: the properties of the ADPase found in *Tm* are similar to those of the ADPase linked to the plasma membranes which exhibits only very low acid phosphatase activity; if we refer to the results of Brightwell and Tappel, the ADPase activity of *Tm* is too high to be responsible for the acid phosphatase content of the preparation; finally tartrate which considerably inhibits the hydrolysis of ADP by the acid phosphatase [11] does not influence ADPase found in *Tm*.

Acknowledgements

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