FURTHER EVIDENCE FOR THE HETEROGENEITY OF LIVER LYSOSOMES

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1. Introduction

Since the discovery of lysosomes [1] it has become evident that the term lysosome, as defined biochemically, covers a wide range of functionally- and morphologically-different particles. Indeed, since lysosomes exhibit a 'life cycle' in functioning as an 'intracellular digestion tract' [2,3], it follows that the population of lysosomes must necessarily be heterogeneous.

However, more recent work has indicated that lysosomes isolated from tissue homogenates may represent different stages of the cycle of a single species of lysosome, and may be enzymically heterogeneous [4,5]. These suggestions have been based on observations of differential distributions of particle-bound lysosomal hydrolases in density-gradient systems, but, as yet, little work has been reported on the isolation and properties of different sub-species of lysosomes from homogenates.

We report here the density distribution of some particulate lysosomal hydrolases from pig liver, and the isolation of a minor lysosomal population with some anomalous properties.

2. Methods and results

Pig liver was obtained from the slaughterhouse and transported to the laboratory in ice. Within 1.5 hr after removal, the liver was homogenised in a Waring blender in ice cold 0.25 M sucrose at full speed for 30 sec. The 1 in 10 (w/v) homogenate was filtered through a single layer of cheesecloth and centrifuged at 1000 *g* (max) for 10 min, to remove nuclei, debris and intact cells. The supernatant suspension was then centrifuged at 15 000 *g* (max) for 20 min to sediment the mitochondrial/lysosomal (ML) fraction. About 20 ml of the ML fraction (1 g protein in 0.25 M sucrose) followed by 20 ml of 0.25 M sucrose overlay was introduced onto a 17–60% (w/v) linear-with-volume sucrose gradient in a BXIV titanium zonal rotor (M.S.E. Ltd.), fitted to an M.S.E. Superspeed 65 centrifuge.

The loading speed was 1500 rpm; this was increased to 47 000 rpm (165 000 *g* max) and the sample centrifuged for 1 hr. The rotor was then unloaded at 1500 rpm by pumping 60% (w/v) sucrose to the periphery, 20 ml factions being collected from the centre line and stored in crushed ice.

The fractions were all diluted 1:1 (v/v) slowly with ice-cold water and then centrifuged at 20 000 *g* (max) for 20 min. The pellets were assayed for total acid phosphatase [6] (EC 3.1.3.2, *p*-nitrophenyl phosphate substrate), aryl sulphatase [7] (EC 3.1.6.1) and β-galactosidase [8] (EC 3.2.1.23) 0.1% (w/v). Triton X-100 being included in all the assay media. The results are shown in fig. 1, where the percentage of the total enzyme activities introduced into the rotor are plotted for each fraction of the gradient. It can be seen that acid phosphatase and aryl sulphatase were localised in a major peak at a density of 1.170 and a minor peak, density 1.050–1.080 corresponding to approximately 6–8% of the total activity of each of these enzymes. β-Galactosidase was present only in the major peak. Comparable results were obtained in four experiments.

The activities measured were entirely sedimentable and thus, unless during the dilution stage lysosomes in different parts of the gradient disrupted to different extents, the measured activities parallel the distribution of lysosomes through the gradient.

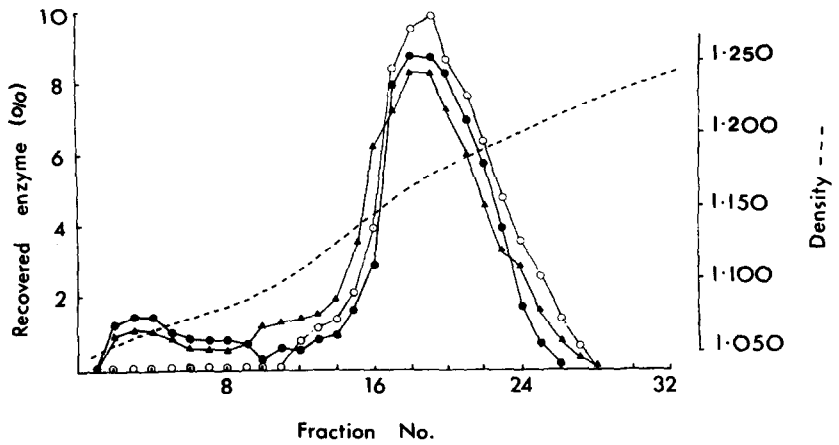

Fig. 1. The isopycnic distribution of paticulate acid phosphatase (▲——▲), aryl sulphatase (●——●), and β-galactosidase (○——○) through a 17–60% (w/v) sucrose gradient in a B X IV zonal rotor. Each fraction collected was diluted 1:1 (v/v) with water and centrifuged at 20 000 *g* for 20 min, and the pellets assayed.

It was noted that the pellets of fractions 2–8 were dark brown, similar to the colour of purified kidney lysosomes [9], whereas pellets of fractions 9–30 were a homogeneous light brown. In view of this, the activities of glutamate dehydrogenase [10] (EC 1.4.1.3), glucose-6-phosphatase [11] (EC 3.1.3.9), and urate oxidase [11], (EC 1.7.3.3), were assayed in each fraction to follow, respectively, the distribution of mitochondria, endoplasmic reticulum, and peroxisomes. Each fraction was diluted to 0.25 M sucrose before assays, as sucrose is known to have an inhibitory effect on these enzymes [12]. For this purpose, the sucrose concentration in each fraction was measured using a Bellingham and Stanley refractometer calibrated directly in percentage sucrose. The distributions of the three enzymes are shown in fig. 2. The mean densities of fractions containing these marker enzymes were similar to those reported for rat liver [13]. The only part of the gradient free of detectable glutamate dehydrogenase and urate oxidase

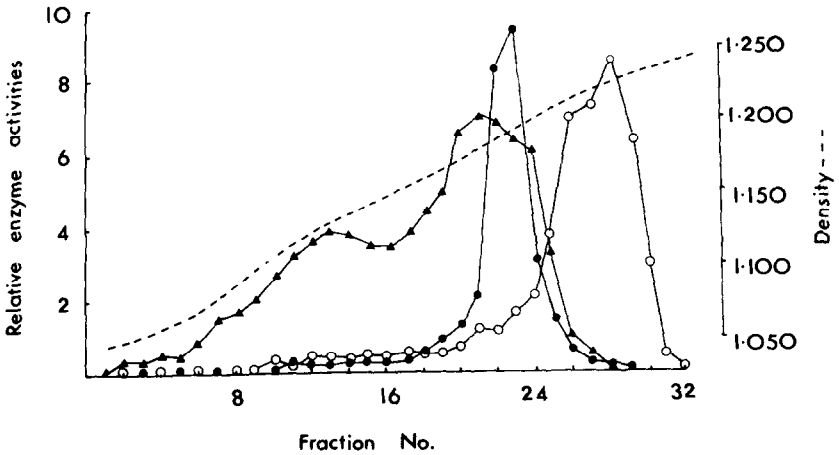

Fig. 2. The isopycnic distributions of glutamate dehydrogenase (●), urate oxidase (○), and glucose-6-phosphatase (▲), through a 17–60% (w/v) sucrose gradient in a B X IV zonal rotor. Each sample was diluted to 0.25 M sucrose before enzymic assay, and the activities are plotted as a relative distribution in arbitrary units.

Table 1
The percentage recoveries of lysosomal hydrolases and other enzymes from a pig liver ML fraction separated by zonal centrifugation through a sucrose gradient: and the relative specific activities of these enzymes in the 'low-density L fraction'. Results are the range for four sets of experiments

Component	Relative specific activity* of the low-density L fraction		% recovery of the total activity** in the original homogenate
	Range	Mean ± S.E.M.	
Acid phosphatase	72–128	98 ± 12	86–96
Aryl sulphatase	45–62	51 ± 10	75–91
Cathepsin D	50–64	59 ±7	82–89
β-Galactosidase	n.d.†	–	75–90
Urate oxidase	n.d.	–	72–81
Glutamate dehydrogenase	n.d.	–	92–104
Glucose-6-phosphatase	0.1–0.17	0.13±0.03	62–75
Protein			79–94

† n.d. = not detectable.
* Calculated as the ratio of the specific activity of the enzyme in this fraction to that in the homogenate.
** Calculated from the sum of the activities in fractions F_{2-8} (supernatant plus pellet) and F_{9-32}, plus the contributions of the nuclear and post-ML fractions, in relation to the activities of the original homogenate.

was fractions 2–8, where the minor lysosome peak (hereafter called the low-density L fraction) was found.

It therefore seemed desirabable to characterise this fraction further by determining the relative specific activities of some lysosomal hydrolases in pooled fractions 2–8.

A zonal centrifugation was carried out as above and fractions 2–8 and 9–32 were pooled to give two bulked fractions designated respectively, F_{2-8} and F_{9-32}. Fraction F_{2-8} was diluted 1:1 (v/v) with ice-cold water and centrifuged at 20 000 *g* (max) for 20 min to seperate pellet from supernatant. Each of these was then adjusted to 0.25 M sucrose and, together with the pooled F_{9-32} (diluted to 0.25 M sucrose), were assayed for acid phosphatase, aryl sulphatase, β-galactosidase, cathepsin D [14] (EC 3.4.4.23), urate oxidase, glutamate dehydrogenase, glucose-6-phosphatase and protein [15]. Corresponding assays were carried out with the inital whole homogenate, the nuclear fraction, and the post-ML supernatant. Relative specific activities (RSA) of the various enzymes were calculated for the pellet from the pooled fraction F_{2-8}. The results are shown in table 1, together with percentage recoveries of the enzymes for the whole fractionation scheme.

3. Discussion

It can be seen that the RSA of hydrolases in pooled F_{2-8} reached the very high values of 45–128, depending on the enzyme assayed. The fraction was devoid of detectable urate oxidase and glutamate hydrogenase activities, and contained only low levels of glucose-6-phosphatase. From these results it was concluded that the low-density L fraction represents a relatively uncontaminated preparation of lysosomes which is enzymically different from the major population in being devoid of detectable β-galactosidase activity.

It is interesting to note that lysosomes in homogenates of *T. pyriformis* form two populations during isopycnic centrifugation. These two populations exhibit an enzyme heterogeneity. The high density lysosomes are rich in carbohydrases, deoxyribonuclease and phosphatase and low in ribonuclease and proteinase. In the low density lysosomes this relation between the various enzyme activities is reversed.

It has previously been calculated that lysosomes constitute only 0.65% of total cellular protein in rat liver [17], and it would thus be expected that the hydrolases in a purified preparation of liver lysosomes would be purified some 150 times over the homogenate. The present results give a value of the RSA of acid phosphatase of 72–128 for the 'low-density L fraction'.

The conclusion that this fraction contained lysosomes and did not represent some artifact of homogenisation was strengthened by the finding that, when assayed under isotonic conditions, the β-glycerophosphatase and aryl sulphatase activities of the low-density L fraction were some 75–85% latent. Also, previously sedimentable activity was released into the supernatant (after 20 000 *g* × 20 min centrifugation) on warming the low-density L fraction at 37°C in 0.25

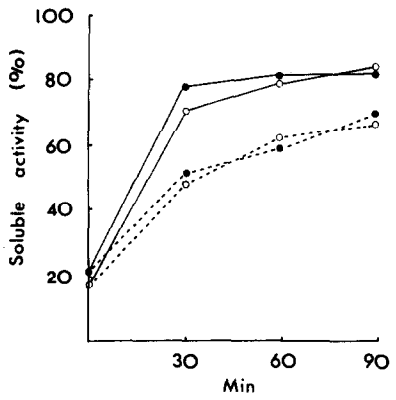

Fig. 3. Release of soluble acid phosphatase (•) and cathepsin D (○) activity from the 'low-density L fraction', suspended in 0.25 M sucrose at 37°C, with (——) and without (– – –) the addition of 2.5×10^{-4} M progesterone. 5 ml of suspension (approx. 1 mg protein/ml) in 0.25 M sucrose was incubated in the presence and absence of 2.5×10^{-4} M progesterone added in 25 µl of ethanol. The same volume of ethanol was added to the controls. At suitable intervals aliquots were removed. The total activities were assayed after dilution of the sample with 10 vol of distilled water, and the soluble activities were assayed in the clear supernatants after centrifugation at 20 000 *g* for 20 min.

M sucrose, and this release was potentiated by the addition of progesterone (a known lysosome-lytic agent) to a final conc. of 2.5×10^{-4}M, as shown in fig. 3.

Suspensions of purified rat kidney lysosomes [9] or polymorphonuclear leucocyte granules [18], show a decrease in absorbance at 520 nm, on lysis, (i.e. as they release soluble enzymes). We measured the absorbance of a suspension of the low-density L fraction at 520 nm using a Perkin–Elmer recording spectrophotometer. Although warming the sample at 37°C for 90 min released some 50% of the cathepsin D of the fraction, no change in absorbance was measured.

Thus it may be that this fraction is anomalous in not exhibiting an absorbance decrease on release of soluble enzymes. Possibly, in this case, the hydrolases are absorbed onto a central matrix as proposed by Koenig [19] in which case an absorbance decrease might not be expected. It will await a true purification of lysosomes from the major peak to determine if this fraction differs from the major population present in pig liver or whether the phenomenon of absorbance decrease on release of soluble enzymes is not a property of liver lysosomes.

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