QUANTITATION OF ACID PHOSPHATASE AND ARYL SULPHATASE IN RAT HEPATIC PARENCHYMAL AND KUPFFER CELLS

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1. Introduction

The two acid hydrolases 'acid phosphatase' (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) and 'aryl sulphatase' (aryl-sulphate sulphohydrolase, EC 3.1.6.1) are both enzymes which are known to be present in the lysosomes of almost all cell types [cf. 1]. Histochemical studies have revealed apparent activity of acid phosphatase in both hepatic parenchymal and Kupffer cells [2,3], and parenchymal liver cells have been claimed to show enzyme activity in granular form – as revealed by light microscopy of sections incubated at pH 5.5 [4]; however, histochemical methods have so far failed to reveal the situation in the Kupffer cells [5]. Evidence from studies of other tissues have suggested that lysosomes are heterogeneous with regard to content of lysosomal enzymes, and it has been claimed – on the basis of results of histochemical studies – that the distribution of aryl sulphatase may not completely agree with the distribution of acid phosphatase [4,6]. Furthermore, higher purification of aryl sulphatase than for instance acid phosphatase was obtained in lysosomes isolated from rat liver [7].

Methods have recently been worked out for the separation of parenchymal and Kupffer cells in highly purified fractions of rat liver [8–10]. In the present study these methods were utilized with the aim of elucidating and comparing the activities of aryl sulphatase and acid phosphatase in the two main cell types of the liver.

2. Materials and methods

Male Sprague Dawley rats weighing 170–200 g were used. The animals were maintained on ordinary laboratory chow and water ad libitum. A heterogeneous liver cell suspension was prepared by perfusing the liver *in vitro* [8,9,11] with 0.05% collagenase (type I) (Sigma) in Hanks' solution for 6–8 min. In accordance with previous results it was found that the presence of Ca^{2+} (4 mM) in the enzyme perfusion medium reduced the time needed to disperse the cells [11]. The liver was pre-perfused without recirculation for 4 min with calcium-free Hanks' solution. During this period liver lobes were removed for the preparation of whole liver homogenates. The perfusion fluid was gassed with 95% O_2 and 5% CO_2 during the entire perfusion; pH was kept constant at 7.45 by the addition of sodium bicarbonate. During perfusion with enzyme 50 ml of collagenase solution was recirculated at constant flow (45 ml/min). The perfusion fluid was gassed vigorously in a cylinder through which the solution was recirculated. A liver cell suspension was prepared by shaking the liver carefully in about 50 ml of ice-cold Hanks' solution containing 30 mM HEPES (Sigma). pH was adjusted to 7.45 by the addition of NaOH. More than 90% of the liver protein was recovered in the cell suspension. About 90% of the cells resisted trypan blue uptake. The cell count was estimated in a Bürker chamber.

Purified *parenchymal cells* were prepared by low-speed centrifugation as described previously [9]. 2% albumin (bovine, crystallized and lyophilized) (Sigma) was included in the medium during centrifugation. About 70% of the hepatocytes in the original

Table 1
Activities of acid phosphatase and aryl sulphatase in rat liver homogenates, parenchymal and Kupffer cells.

	Liver homogenate enzyme activity[1] /mg protein	Kupffer cells enzyme activity /mg protein	/10^6 cells	Parenchymal cells enzyme activity /mg protein	/10^6 cells
Acid phosphatase	25.9 ± 1.0	40.0 ± 2.8	6.10 ± 0.2	19.6 ± 0.2	43.8 ± 0.4
Aryl sulphatase	8.32 ± 0.49	72.2 ± 7.9	11.0 ± 0.8	5.89 ± 0.28	13.0 ± 0.7

[1] Enzyme activity is expressed as μmole $\times 10^{-3}$ PO_4^{--} (acid phosphatase) or as μmole $\times 10^{-3}$ 4-NC (aryl sulphatase) released/min at 37°C.
Presented data are expressed as means ± SEM and are the results of at least five experiments.

cell suspension was recovered in the final pellet, and less than 2% of the cells were nonparenchymal. About 97% of the purified parenchymal cells resisted trypan blue uptake.

Kupffer cells were prepared by incubating portions of original cell suspensions with 0.25% pronase (grade B) (Calbiochem) in Hanks' medium buffered with 30 mM HEPES. This method is based on that of Mills and Zucker-Franklin [10], but the use of cell suspensions rather than tissue slices was found to give higher and more reproducible yields of Kupffer cells. 25 ml portions of the original cell suspensions were incubated at 37°C during shaking (100 cycles/min) for about 90 min; after this time period virtually all hepatocytes were selectively destructed by pronase. Purified Kupffer cells were then prepared by centrifuging the incubation mixture 4 times at 500 *g* for 3.5 min. Ninety-five to one hundred per cent of the purified Kupffer cells were viable by the trypan blue exclusion test. About 70% of the Kupffer cells in the original cell suspension was recovered in the Kupffer fraction; this represents about 20% of the total number of cells in the original suspension.

Purified hepatocytes and Kupffer cells were resuspended and washed twice in 0.25 M sucrose. The final cell concentration was about 2.5 × 10^6 cells/ml. The cells, as well as the liver tissue, were homogenized in 0.25 M sucrose by use of Dounce homogenizers with tightly fitting pestles. 0.1% (v/v) Triton X-100 was included in the homogenates to assure that total enzyme activity was measured.

The activity of acid phosphatase (EC 3.1.3.2) was estimated at pH 5.1 according to Berthet and de Duve [12] as modified by Bowers et al. [13]. Aryl sulphatase (EC 3.1.6.1) was assayed at pH 5.6 as described originally by Roy [14] and further modified by Bowers et al. [13]. Protein was measured by using the method of Lowry et al. [15].

3. Results and discussion

Table 1 shows the activities of acid phosphatase and aryl sulphatase in the whole liver homogenate and purified hepatic parenchymal and Kupffer cells. It will be seen that the enzyme activity 'per cell' is about the same in purified parenchymal and Kupffer cells as regards aryl sulphatase; on the other hand, the activity of acid phosphatase is about 7 times higher in the hepatocytes. The specific activity (estimated on a protein basis) in the Kupffer cell fraction of aryl sulphatase is more than 10-fold – and of acid phosphatase about 2-fold – than in the parenchymal cell fraction. The specific activities of aryl sulphatase and acid phosphatase in the whole liver homogenate are somewhat higher than those recorded in the isolated parenchymal cells.

These findings show: a) that both Kupffer and parenchymal cells in rat liver contain aryl sulphatase; b) that the Kupffer/parenchymal cell ratio concerning the specific activities of aryl sulphatase is much higher than corresponding ratio for another lysosomal enzyme, acid phosphatase. Other studies have demonstrated that the ratio for β-glucuronidase and acid DNAase is also lower than that for aryl sulphatase [16]. This indicates that among the lysosomal enzymes studied so far, aryl sulphatase is the enzyme most heavily concentrated in the Kupffer cells.

The reason for this selectively high sulphatase activity in the Kupffer cells is not readily apparent. The biological function of aryl sulphatase is enigmatic, but it has been suggested that this enzyme is involved

in the hydrolysis of cerebroside-3-sulphate [17]. Rappay et al. [18] propose that non-specific aryl sulphatase localized in the lysosomes may participate in the modulation of the 'specific steroid-sulphurulation-desulphurulation process'. Lowered activity of aryl sulphatase A seems to be the reason why sulphated glycolipids accumulate in the lysosomes of various tissues in patients with metachromatic leucodystrophy [19]. It is of interest to note in this connection that the Kupffer cells appear to be able to accumulate bile pigments in their lysosomes [20] and participate in the metabolism of bilirubin [21]; this metabolism seems to involve sulphation of bilirubin [22]. Perhaps the Kupffer cells have a specific and unique function in this type of conjugation of bilirubin, while the parenchymal cells perform glucuronidation.

In conclusion, the Kupffer cells in rat liver show exceptionally high activity of aryl sulphatase in comparison with other lysosomal enzymes.

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