Effect of Chemical, Physical and Enzymic Treatments on Lysosomes from AH-130 Yoshida Ascites Hepatoma

Available evidence indicates the existence of differences between some physical properties of tumour and liver lysosomes[1,2], suggesting a different structural state of membranes of particles from the two tissues. Data in this paper accord with this suggestion and provide evidence of differences in structural organization of some constituents of the two types of lysosomes.

Materials and methods. Livers of 24 h fasted Long-Evans rats (200–250 g) have been suspended in cold 0.29 *M* sucrose, 0.02 *M* *Tris*-Cl, pH 7.4, 0.002 *M* *Tris*-EDTA, and 2‰ bovine serum albumin (isotonic sucrose). Homogenates have been prepared with 3 strokes at 1500 rpm, in a Potter-Elvehjem homogenizer, provided with a loosely fitting pestle. Homogenates from 5 days inocules in Long-Evans rats, of AH-130 Yoshida ascites hepatoma, have been made in a similar way[3]. Both preparations have been centrifuged at 6000 *g*-min, supernatants are referred to as 'homogenates'. Liver and hepatoma L fractions have been prepared according to De Duve et al.[4]. Determinations of acid phosphatase (*orto*-phosphoric monoester phosphohydrolase, EC. 3.1.3.2.) and β-glucuronidase (glucuronide glucuronohydrolase, EC. 3.2.1.31) in 'homogenates' and L fractions, have been performed according to Appelmans et al.[5] and Gianetto et al.[6]. Proteins have been determined by a biuret procedure[7].

Results and discussion. Figure 1 shows that the activation of acid phosphatase (APase) and β-glucuronidase (β-GNase) induced at pH 5 and 37 °C, or by hypotonic treatment, is lower in hepatoma than in liver 'homogenates'. After 75–90 min incubation at pH 5 and 37 °C, free activity of enzymes in hepatoma 'homogenates' is 50–55% of the one measured in the presence of Triton (Figures 1 A and B). No more increase of activity is obtained by prolonging the incubation to 120 min or longer, although Triton is still able to induce further 40% activation of both enzymes (not shown). When hepatoma 'homogenates' are submitted to hypotonic conditions, no more than 60% enzyme activity release is obtained in the range of the osmolarities employed (Figures 1 C and D). Activation is complete if hypotonic treatment is performed at 37 °C.

These data confirm the results obtained by Horvat and Touster[1] with L + M fraction from Ehrlich ascites adenocarcinoma. As concerns their evaluation, the possibility should be considered that differences in osmotic

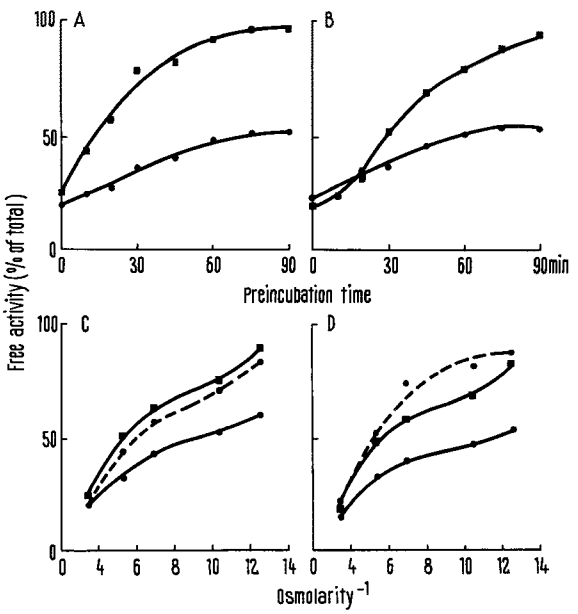

Fig. 1. Thermal and hypotonic activation of lysosomes ('homogenates') of normal liver and Yoshida ascites hepatoma. (A) and (B), aliquots of 'homogenates' (1 mg protein) in isotonic sucrose were added to a reaction medium containing 0.29 *M* sucrose and 0.05 *M* acetate buffer, pH 5, and preincubated at 37 °C. APase and β-GNase activities were started by addition of substrates and were determined according to [5] and [6]. (C) and (D), aliquots of 'homogenates' were added to a reaction mixture containing, in 1 ml, 0.02 *M* *Tris*-Cl, pH 7.4, and different amounts of sucrose to obtain derived osmolarities. Osmolarity was defined as analytical molar concentration multiplied by the number of osmotically active components. After 30 min at 0 °C, suspensions were brought to isotonic conditions adding sucrose, and employed as source of enzymes. Curves in (A) and (C) refer to APase activity, curves in (B) and (D) to β-GNase activity in liver (■) and hepatoma (●). Dotted curves refer to samples incubated during 10 min at 37 °C in hypotonic conditions. Free activity is measured as percent of total activity in samples treated with 0.1% Triton X-100. Each point represents average value of 6 experiments for liver and hepatoma homogenates.

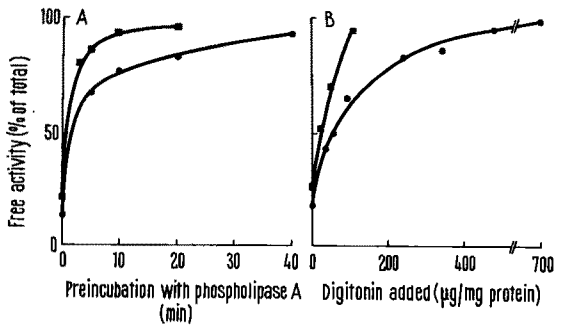

Fig. 2. Activation of lysosomes (L fraction) of normal liver and Yoshida ascites hepatoma, with phospholipase A and digitonin. (A), L fractions were preincubated 10 min at 37 °C in 0.29 *M* sucrose, 0.02 *M* *Tris*-Cl, pH 7.4, 2‰ bovine serum albumin, 0.005 *M* $CaCl_2$, and 0.005 U/mg of phospholipase A (Sigma, from *Crotalus adamanteus*). At the times indicated aliquots of suspensions (0.7–1 mg protein) were added to reaction mixtures for the determination of β-GNase. (B), L fractions in isotonic sucrose, were incubated 20 min at 0 °C with the indicated amounts of digitonin (twice recrystallized from ethanol). After incubation, enzymic activity was determined on aliquots of suspensions as in (A). Free activity is measured as percent of total activity in Triton treated samples. Each point represents average value of 4 experiments in (A) and 5 experiments in (B) for liver (■) and hepatoma (●) lysosomes.

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behaviour or in the surface of particles from different tissues, may influence the response to hypotonic conditions or to lytic enzymes activated at pH 5 and 37 °C. The possibility that volume and not structural differences are concerned in the observed phenomena has been checked by submitting liver and hepatoma lysosomes (L fractions) to phospholipase A (phosphatide acyl-hydrolase, EC. 3.1.1.4), digitonin and trypsin. The response to these agents may be influenced by extra lysosomal components or lysosomal volume, so that apparent resistance is dissimulated. Increasing amounts of added agents, however, may overcome lysosomal apparent resistance. In the absence of this overcoming effect, the role of lysosomal membrane properties must be taken into account.

When liver and tumour L fractions are submitted to phospholipase A (Figure 2A), the β-GNase activation in tumour L fraction proceeds more slowly but, practically, to the same extent as in liver lysosomes. Digitonin, also, is able to liberate all the β-GNase activity in hepatoma lysosomes, when added in 3 times the amount which induces almost complete activation of liver lysosomes enzymic activity (Figure 2B). Different patterns are observed when hepatoma and liver lysosomes are treated with trypsin. Preincubation for 30 min at 37 °C of lysosomes from both tissues with 100 μg of trypsin per mg protein fails to induce enzyme maximum activation (Figure 3A). Once again tumour lysosomes are less susceptible to the damaging agent. It appears that after 10 min incubation only a slight increase in activity is obtained, although Triton is still able to induce enzyme release up to 98%. Figure 3B shows that further activation up to 87% of liver lysosomes β-GNase is obtained with 400 μg of trypsin per mg protein. This activity can no longer be activated by Triton, probably because of β-GNase partial inhibition. In the case of hepatoma L fraction, 40% maximum activation is induced by trypsin and no additional increase of activity occurs between 100 μg and 1200 μg of trypsin per mg protein. Amounts of trypsin higher than 200 μg per mg protein induce some β-GNase inhibition. This inhibition accounts for the difference between free activity calculated as percent of total activity, or of that released by Triton after trypsin treatment.

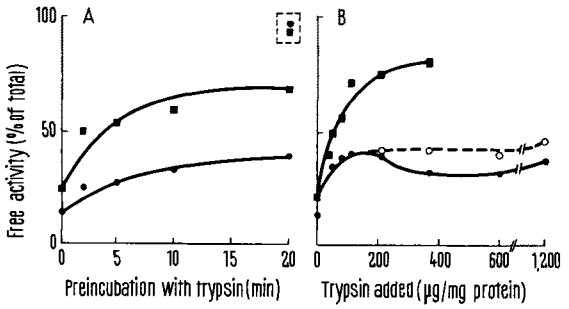

Fig. 3. Activation by trypsin of lysosomes (L fraction) of normal liver and Yoshida ascites hepatoma. (A), aliquots of L fractions (0.7–1 mg protein) were preincubated at 37 °C for varying perdiods with trypsin (Sigma, type III, 100 μg/mg), and β-GNase was determined. (B), incubation with different amounts of trypsin was made 20 min at 37 °C. Other conditions as in (A). Free activity is calculated as percent of total activity in Triton treated samples. Each point represents an average value of 5 and 6 experiments, respectively, in (A) and (B), for liver (■) and hepatoma (●) lysosomes. Symbols in dotted square indicate Triton-induced activation after incubation with trypsin. Dotted line refers to free activity calculated as percent of Triton-induced release after preincubation with trypsin.

The slower response of tumour lysosomes to digitonin and phospholipase A may depend on quantitative differences of membrane constituents attacked by these agents, as well as of the whole L fraction chemical composition. The latter mechanism does not explain the inability of increasing amounts of trypsin to induce activation of β-GNase of hepatoma lysosomes. Differences in molecular organization or in accessibility to trypsin of protein in lysosomal membranes could be concerned in the phenomenon. In this connection it is of interest that also the damaging effect of endogenous lysosomal enzymes, activated by incubation at pH 5 and 37 °C, reaches a saturation point when it can no longer be activated by prolonging the incubation time. These patterns could account for different structural organization in lysosome membranes of hepatoma and liver particles[8].

Riassunto. Omogenati e frazioni L di epatoma ascite di Yoshida AH-130 sono più resistenti di quelli di fegato all'attivazione termica a pH 5, al trattamento ipotonico ed alla digestione con tripsina.

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Release of Growth Hormone from Somatotropin Producing Cells of Hepatectomized Mice Without the Participation of the Golgi Complex[1]

Somatotropin producing cells of hepatectomized mice present striking changes suggesting intense hormone synthesis and release, some hours before the first DNA synthesis peak in the regenerating liver[2,3].

We have not found in these cells the ultrastructural picture of secretion previously described for somatotropin producing (STH) cells in different situations[4–6], in which the outstanding mechanism is that of exocytosis[7]. Alternatively, images such as those described by SCHARRER[8] for neurosecretion in Blattarian insects, were frequently found in our hepatectomy STH cells.

STH cells of hepatectomized mice present strong cartographic dilatation of the endoplasmic reticulum, containing dense material[2]. The dilated cisternae were frequently found contacting with the plasmalemma in zones of increased electron density (Figures 1 and 2).

These images suggest a direct release of the hormone synthesized by the endoplasmic reticulum, bypassing the