COMPARATIVE BINDING OF ALBUMIN AND β-GLUCURONIDASE BY RAT LIVER MICROSOMES

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SUMMARY Rat liver microsomes, free of lysosomal β-glucuronidase, were subjected to sonication. Under the experimental conditions used, 95 % of the microsomal β-glucuronidase activity was solubilized while only 11 % of the albumin was released in the soluble fraction. The results indicate that microsomal β-glucuronidase is not contained in the cisternae of the microsomal vesicles but is attached to the membranes by bonds that are broken by sonication before the membranes are disrupted.

INTRODUCTION The intracellular pathway of the secretory proteins has been well established in a few glands. Histological and biochemical studies performed on the pancreas by Caro and Palade (1) and Jamieson and Palade (2,3) show that zymogen proteins synthesized by bound ribosomes gain access to the cisternae of the rough endoplasmic reticulum then through the cisternae of the smooth endoplasmic reticulum are transported to the Golgi apparatus. From the Golgi the zymogen granules move to the periphery of the cell and are finally secreted.

Recently Peters et al. (4) and Redman and Cherian (5) showed by biochemical studies that the newly synthesized albumin follows a similar pathway before being secreted by the hepatocyte.

Novikoff and co-workers (6) by means of histological evidence postulated that the formation and the intracellular transport of liver lysosomal hydrolases are similar to those of the zymogens of the pancreas. According to these authors the vesicles detaching from the Golgi apparatus would be primary lysosomes.

According to Kato et al. (7) mouse kidney β-glucuronidase is transported from the endoplasmic reticulum where it is synthesized to the lysosomes. Van Lancker and Lentz (8) have published similar results for rat liver β-glucuronidase.

However it is not known if the newly formed β-glucuronidase remains attached to the membranes of the endoplasmic reticulum or passes directly into the cisternae while on its pathway to the lysosomes. Since it is generally accepted that the ultrasonic treatment of liver microsomes releases in a soluble form the intravesicular albumin by rupturing the microsomal vesicles (9), we thought that by comparing the release of albumin to that of β-glucuronidase from sonicated microsomes, some information could be obtained on the intramicrosomal localization of β-glucuronidase.

We have found that under the experimental conditions used, sonication released 95 % of the β-glucuronidase activity in a soluble form while only 11 % of the albumin was released from rat liver microsomes.

METHODS AND RESULTS Sprague-Dawley male rats weighing 125-150 g were fasted for 16 h and killed by decapitation. Liver microsomes were prepared according to the method of DeDuve et al. (10) except that the mitochondrial and lysosomal fractions were centrifuged together and washed once with 0.25 M sucrose. The supernatant from this washing was added to the supernatant of the mitochondria and lysosomes centrifugation. The microsomes were centrifuged from these combined supernatants and washed twice with distilled water to solubilize contaminating lysosomal β-glucuronidase. They were then resuspended in 0.15 M NaCl using a Dounce homogenizer with a loose fitting pestle. We have shown in a previous publication (11) that microsomes prepared according to the above method are free of lysosomal β-glucuronidase.

Microsomes from 5 g of liver suspended in 50 ml of 0.15 M NaCl were placed in a container cooled by circulating water at 4° and treated

by ultrasonic vibrations. A Biosonik II apparatus (Bronwill Scientific, Rochester, N.Y., U.S.A.) was used at half the maximum intensity.

During the ultrasonic treatment, aliquots of 10 ml were removed at 5, 10, 20 and 30 minutes and centrifuged at 105,000 x g for 30 min. The percentage of solubilized β-glucuronidase, glucose-6-phosphatase, protein and serumalbumin were determined in the clear supernatants. The respective 100 % values were given by a sample of the sonicated but uncentrifuged microsomal suspension. Glucose-6-phosphatase was determined according to the method of DeDuve et al. (10), β-glucuronidase by the method of Gianetto and DeDuve (12), and proteins by the method of Lowry et al. (13). Serumalbumin was determined in the supernatants and in the microsomal suspensions by the immunological method of Sargent (14), the serumalbumin of the microsomal suspensions being at first solubilized by sodium deoxycholate (DOC) treatment (0.5 % final concentration, 50 mg DOC per g of liver) according to the method of Peters (15).

After 30 min. of ultrasonic treatment the pellet obtained by centrifugation (105,000 x g for 30 min.) was treated with DOC and assayed for serumalbumin as above. The recovery of serumalbumin was 96 %; 85 % being found in the centrifugation pellet and 11 % solubilized by sonication.

Suitable tests were made to verify that the activities of β-glucuronidase and glucose-6-phosphatase were not modified by the ultrasonic treatment. Recoveries ranging from 90 to 100 % were obtained in all cases. Figure 1 shows the results of five experiments.

DISCUSSION By sonicating microsomes Glauman solubilized 87 to 90 % of the serumalbumin which is contained in the microsomal cisternae (16). As can be seen in Figure 1, only 11 % of the serumalbumin was released by sonication of the microsomes. This result indicates that under our experimental conditions the microsomal membranes were only slightly rup-

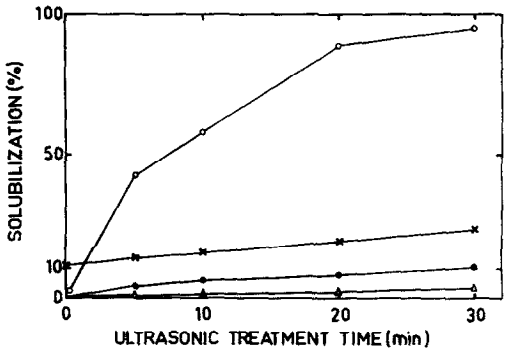

Figure 1 : Solubilization of β-glucuronidase ○, serumalbumin ●, proteins ×, and glucose-6-phosphatase △, by sonication of a microsomal suspension in 0.15 M NaCl.

tured. That the membranes were not heavily damaged is also shown by the fact that only 12 % of the proteins were solubilized by sonication. This 12 % resulting from the difference between the percentage of total proteins released after 30 min. of sonication (23 %) and the percentage released from nonsonicated microsomes (11 %).

However, even if the membranes were not sufficiently ruptured to permit the total release of serumalbumin from the microsomal cisternae, the β-glucuronidase was almost totally solubilized. This shows that the microsomal β-glucuronidase is not contained in the cisternae but is probably attached to the membranes. Its mode of attachment seems however quite different from that of glucose-6-phosphatase, a microsomial membrane constituent (17), of which only a negligible amount was released by sonication.

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