DIFFERENCE IN ELECTROPHORETIC MOBILITY BETWEEN THE LYSOSOMAL AND THE MICROSOMAL β-GLUCURONIDASE OF RAT LIVER

L. Mameli, M. Potier and R. Gianetto

Département de Biochimie, Université de Montréal, Montréal, Canada

Received November 8, 1971

SUMMARY -- Crude, soluble β-glucuronidase (EC 3.2.1.31) preparations from rat-liver lysosomes and microsomes were submitted to disc electrophoresis on polyacrylamide gel. Under the experimental conditions used the lysosomal β-glucuronidase migrates faster than the microsomal enzyme.

INTRODUCTION -- Several authors have shown by tissue fractionation studies (1) and by histochemical techniques (2) that rat-liver β-glucuronidase has a dual localization, being associated with the lysosomes and the endoplasmic reticulum.

Kato et al. (3) have shown recently that in mouse kidney β-glucuronidase is a glycoprotein that is transported from the endoplasmic reticulum where it is synthesized to the lysosomes. Van Lancker and Lentz (4) have published similar results for rat liver and have shown that the lysosomal enzyme has the same electrophoretic mobility on polyacrylamide gel as the microsomal enzyme solubilized by treating the microsomal fraction with ribonuclease.

Comparative studies undertaken in our laboratory between the microsomal β-glucuronidase and the lysosomal enzyme required a method to ascertain the non-contamination of the microsomal fraction by the lysosomal β-glucuronidase.

We have found that the β-glucuronidase solubilized by ultrasonic vibrations from the twice washed microsomal fraction has a different electrophoretic mobility on polyacrylamide gel than the lysosomal enzyme and is not contaminated by it.

METHODS AND RESULTS -- Livers were obtained from male Sprague-Dawley rats (125-150 g), fasted for 16 h and killed by decapitation. Homogenization and differential centrifugation of the homogenate were performed according to the method of De Duve et al. (1), except that the mitochondrial and lysosomal fractions were centrifuged together and washed once with 0.25 M sucrose.

The pellet containing mitochondria and lysosomes was then resuspended in cold distilled water (10 ml/g of liver), treated during 2 min in a precooled Waring Blendor and centrifuged at 105,000 x g during 30 min. The clear supernatant contained the soluble lysosomal β-glucuronidase.

Microsomes were washed twice with distilled water, which solubilized the contaminating lysosomal β-glucuronidase by osmotic shock, and then resuspended in 0.15 M NaCl (2 ml/g of liver). The microsomal suspension was then sonicated at 4° for 20 min at half the maximum intensity, using a Biosonik II apparatus (Bronwill Scientific, Rochester, N.Y., U.S.A.). A clear supernatant containing 80-90 % of the microsomal β-glucuronidase was obtained by centrifugation of the sonicated suspension at 105,000 x g for 30 min.

The β-glucuronidase activity was determined in all preparations according to the method of Gianetto and De Duve (5).

Disc electrophoresis was performed on polyacrylamide gel according to the procedure of Davis (6). The β-glucuronidase activity, after electrophoresis, was detected by the following procedure. The gel was incubated at 37° in 0.2 M acetate buffer, pH 4.0, containing 0.5 M NaCl, 0.003 M 8-hydroxyquinoline β-D-glucuronide as the substrate and

Fast Garnet GBC, 1 mg/ml, as the diazo coupling agent. The enzyme was revealed as a brown band. Three gel columns were prepared as follows : one containing a sample of the microsomal β-glucuronidase, the second containing a sample of the lysosomal β-glucuronidase, and the third containing a mixture of both enzymes. A Chromoscan apparatus (Joyce, Loebl and Co. Ltd., Princesway, England) was used to measure the intensity of the coloured bands resulting from the β-glucuronidase activity. The results are shown in Fig. 1.

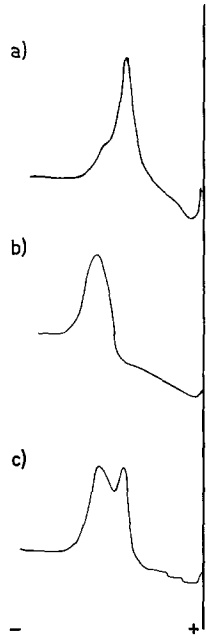

Figure 1 : Scanning of the polyacrylamide gels after electrophoresis. a) Microsomal β-glucuronidase, b) lysosomal β-glucuronidase, c) mixture of microsomal and lysosomal β-glucuronidase.

DISCUSSION -- The results presented in this report show that the electrophoretic mobility of the lysosomal β-glucuronidase is different from that of the microsomal β-glucuronidase. Electrophoretic separation is possible when both enzymes are present in the same preparation.

Our results differ from those presented by Van Lancker and Lentz (4), who dissolved the microsomal enzyme by incubating the microsomal fraction with large amounts of ribonuclease. It is possible that the β-glucuronidase solubilized by this procedure differs from the one solubilized by sonication. The β-glucuronidase solubilized by our procedure may perhaps have ribonucleic acids attached to it. Further studies are in progress to clarify this point and purify the microsomal β-glucuronidase.

ACKNOWLEDGMENT -- This research was supported by a grant from the Medical Research Council of Canada.

REFERENCES

1. DeDuve, C., Pressman, B.C., Gianetto, R., Wattiaux, R., and Appelmans, F., Biochem. J., 60, 604 (1955).
2. Fishman, W.H., Goldman, S.S. and De Lellis, R., Nature, 213, 457 (1967).
3. Kato, K., Ide, H., Shirahama, T. and Fishman, W.H., Biochem. J., 117, 161 (1970).
4. Van Lancker, J. and Lentz, P.L., J. Histochem. Cytochem., 18, 529 (1970).
5. Gianetto, R. and DeDuve, C., Biochem. J., 59, 433 (1955).
6. Davis, B.J., Ann. N.Y. Acad. Sci., 121, 404 (1964).