DIFFERENTIAL UPTAKE OF HUMAN β-GLUCURONIDASE ISOENZYMES FROM SPLEEN BY DEFICIENT FIBROBLASTS

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SUMMARY

Two fractions of β-glucuronidase have been separated from an extract of human spleen by salt elution from DEAE. The two fractions correspond to two bands on gel acrylamide electrophoresis at pH 4.3. The 0.02 M NaCl eluate gives an immunodiffusion reaction of identity with β-glucuronidase purified from liver, and is taken up poorly by deficient human fibroblasts. The 0.1 M eluate fails to cross react with the antibody to the liver enzyme, and is taken up avidly by deficient fibroblasts.

INTRODUCTION

Several lysosomal hydrolases have been shown to be taken up by fibroblasts in tissue culture (1-6). Brot et al. examined β-glucuronidases from different tissues and found a marked difference in the efficiency of their uptake by deficient fibroblasts in vitro (7). We have separated a high and a low uptake form of β-glucuronidase from human spleen and have made some preliminary observations on the two enzymes.

MATERIALS AND METHODS

Enzyme was prepared from the spleen of a 54-year-old male with atherosclerotic heart disease obtained at autopsy. The spleen showed no gross or microscopic abnormalities other than acute congestion. The spleen was homogenized in 2 volumes of 0.5% sodium desoxycholate, and the homogenate dialyzed against 5 mM Tris buffer, pH 8.0. The 100,000 g supernatant constituted the crude extract. The two major fractions of enzyme activity were separated by sequential elution of the enzyme from DEAE with 0.02 M NaCl and 0.1 M NaCl in 5 mM Tris buffer, pH 8.0 (Fig. 1). The fraction eluted with 0.1 M NaCl was rechromatographed on DEAE in a linear gradient 0 to 0.1 M NaCl in Tris buffer.

Gel electrophoresis was carried out at pH 4.3 according to Reisfeld

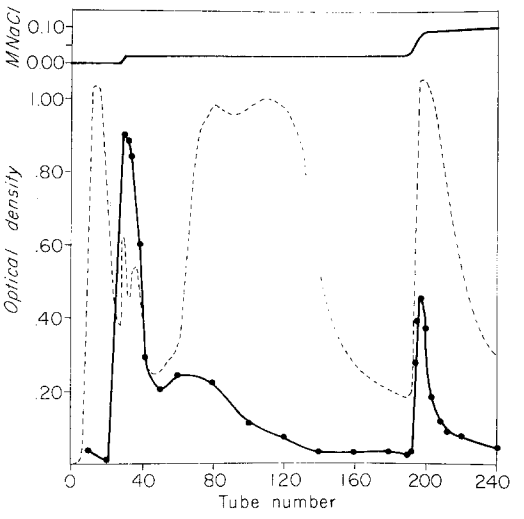


Fig. 1. DEAE chromatography of spleen extract. Spleen was homogenized in 0.5% desoxycholate and applied to DEAE in 5 mM Tris buffer, pH 8.0. Two steps of NaCl concentration were used to elute the column. ------ Protein concentration measured as optical density at 280 nm; ●——● enzyme activity measured as optical density at 420 nm with p-nitrophenyl β-D-glucuronide as substrate.

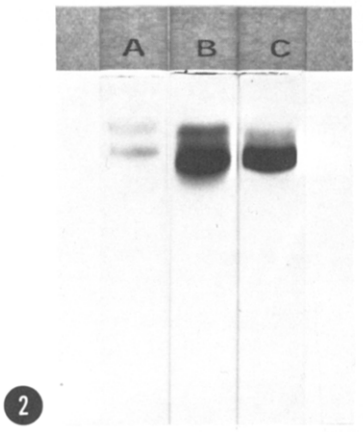
et al. (8), with the exception that no stacking gel was used. Ammonium persulfate was removed by electrophoresis before electrophoresing the enzyme extract. Samples were standardly electrophoresed for 3 hours at 6 ma/gel at room temperature. Enzyme was visualized using Naphthol AS-BI-β-D-glucuronide as substrate and post-coupling with Fast Garnet GBC (9).

β-Glucuronidase-deficient cell culture procedures and assay of uptake of enzyme were carried out as previously described (6).

During enzyme purification, activity was assayed with p-nitrophenyl β-D-glucuronide. 4-Methylumbelliferyl glucuronide was used as substrate for assay of uptake in the tissue culture experiments. Protein was determined by the method of Lowry et al. (10).

RESULTS

Human liver β-glucuronidase purified essentially by the method of Musa et al. (11) gave a single band of enzyme activity on gel acrylamide electrophoresis at pH 4.3. Antibody raised in rabbits gave a single precipitin line by gel diffusion, even though a small second band of protein lacking enzyme



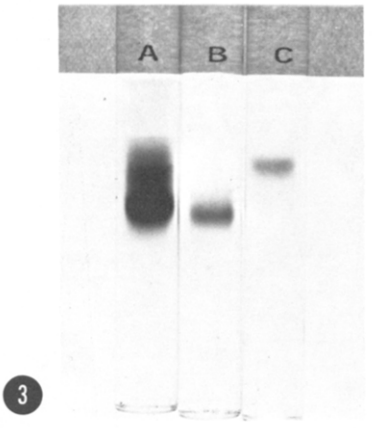


Fig. 2. Acrylamide gel electrophoresis of 0.5% desoxycholate extracts at pH 4.3. Gels were stained for β-glucuronidase activity with Naphthol AS-BI-β-D-glucuronide and Fast Garnet GBC. A - platelets; B - spleen; C - liver.

Fig. 3. Acrylamide gel electrophoresis (pH 4.3) of β-glucuronidase fractions from human spleen. Gels were stained for β-glucuronidase activity. A - Tris buffer dialyzed spleen extract; B - 0.02 M NaCl DEAE eluate; C - 0.1 M NaCl DEAE eluate.

activity was present by gel electrophoresis in the most highly purified enzyme preparation. Staining of the gel diffusion pattern showed that the precipitin band contained active enzyme. Desoxycholate (0.5%) extracts of liver, spleen and platelets each yielded two bands on gel electrophoresis (Fig. 2). The fast band from spleen, which corresponded in migration to the enzyme prepared from liver, was obtained by elution from DEAE with 0.02 M NaCl; the slow band was eluted with 0.1 M NaCl (Fig. 3).

The antibody prepared against highly purified liver β-glucuronidase gave an immunodiffusion pattern of identity with the spleen 0.02 M eluate but no precipitin line with the 0.1 M eluate. Estimates of molecular weight by gel electrophoresis according to the method of Hedrick and Smith (12) showed no difference between the β-glucuronidase activity in the 0.02 M eluate and that in the 0.1 M eluate.

The 0.02 M and 0.1 M eluates of spleen β-glucuronidase were concentrated to approximately equal activity per milliliter by ultrafiltration, sterilized by Millipore filtration and added to cultures of β-glucuronidase-deficient

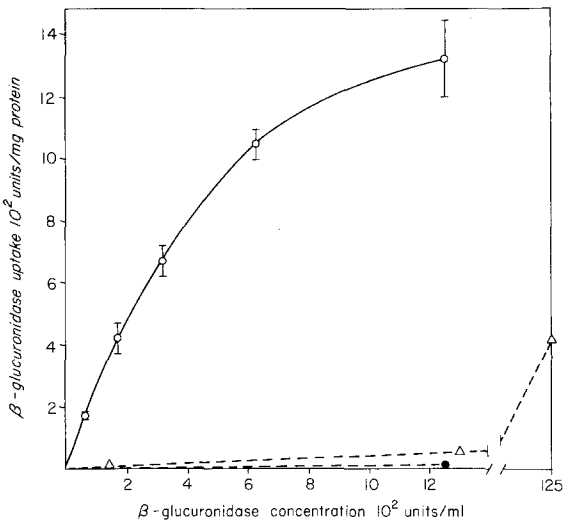


Fig. 4. Uptake of β-glucuronidase by deficient fibroblasts. The enzyme was added to the tissue culture medium, and cells cultured for 24 hours (6). Enzyme activity was measured with 4-methylumbelliferyl β-D-glucuronide. Total cell protein was measured by the method of Lowry et al. (10). ○—○ Mean values of fibroblast-associated enzyme activity ± SE from 3 separate experiments using the 0.1 M NaCl eluted component of spleen β-glucuronidase. Δ----Δ Mean of two values of enzyme activity using β-glucuronidase from liver (6). ●----● Mean value of enzyme activity from 3 separate experiments using 0.02 M NaCl eluted component of spleen β-glucuronidase. One unit of enzyme activity equals 1 mole of substrate hydrolyzed per hour. (Units used previously (6) are incorrect and must be multiplied by 2.5 x 10^{-3}.) The ratio of uptake of the two forms of spleen β-glucuronidase at an initial concentration of 1.25 x 10^3 units/ml in the media was 220:1.

fibroblasts. The β-glucuronidase in the 0.1 M eluate was taken up avidly. The 0.02 M eluate was taken up far less efficiently (Fig. 4). Mixing the two fractions in equal parts had no inhibitory effect on the uptake of the 0.1 M fraction. Uptake of the β-glucuronidase eluated at 0.1 M appeared to approach saturation at the highest concentration tested (Fig. 4).

DISCUSSION

The behavior of the β-glucuronidase eluted from DEAE at 0.1 M (β-glucuronidase - 0.1 M) corresponds in efficiency of uptake to the high uptake enzyme derived from human platelets by Brot et al., while the activity eluted at 0.02 M (β-glucuronidase - 0.02 M) resembles the liver enzyme we have previously described.

β-Glucuronidase isoenzymes have been extensively studied in several animal species: mouse (13), rabbit (14), rat (15, 16) and cow (17); isoenzymes of human β-glucuronidase have been described for liver (18), urine (19) and placenta (20). In the mouse the evidence of Paigen and his coworkers (13) strongly indicates a basic commonality of the multiple forms of β-glucuronidase, and the multiple forms of β-glucuronidase in rabbit liver exhibit immunologic identity (14). The failure of antibody to human liver β-glucuronidase to cross-react with β-glucuronidase - 0.1 M and the immunologic studies of Higashino et al. (18) in which only 61% of spleen β-glucuronidase was precipitable with antibody to the major liver enzyme point to a different relationship between the two human β-glucuronidase forms.

Gel electrophoresis demonstrates that there is a larger proportion of the slow band of β-glucuronidase in platelets, corresponding to β-glucuronidase - 0.1 M, than in spleen; and spleen in turn contains a larger proportion of the slow band than crude extracts of liver. The substantial content of the slow electrophoretic mobility isoenzyme in platelets together with the observation of Brot et al. that platelet enzyme is a high uptake form supports the identification of the slow band as the high uptake form.

The property of the β-glucuronidase with the slower electrophoretic mobility that accounts for its high uptake is not known. Large differences in uptake of glycoprotein by hepatic parenchymal cells have been related to the specific sugar moieties at the non-reducing terminus of the carbohydrate chains (21), and Hickman et al. have been able to markedly depress uptake of N-acetyl-β-D-hexosaminidase by periodation of the enzyme (22). It seems unlikely, but possible, that the antigenic difference between the high and low uptake forms of spleen β-glucuronidase is solely attributable to a difference in carbohydrate components. It is clearly necessary to obtain purified preparations of both forms in order to adequately define the difference between the two forms and thus to identify the basis for the high uptake property. Brot et al. (7) have already pointed out the potential importance of the high uptake form for therapeutic trials.

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REFERENCES

1. Porter, M. T., Fluharty, A. L., and Kihara, H. (1971) Science 172, 1263-1265.
2. Weissman, U. N., Rossi, E. E., and Herschkowitz, N. N. (1972) Acta Paediat. Scand. 61, 296-302.
3. Bach, G., Friedman, R., Weissmann, B., and Neufeld, E. F. (1972) Proc. Nat'l Acad. Sci. 69, 2048-2051.
4. Hall, C. W., Cantz, M., and Neufeld, E. F. (1973) Arch. Biochem. Biophys. 155, 32-38.
5. O'Brien, J. S., Miller, A. L., Loverde, A. W., and Veath, M. L. (1973) Science 181, 753-755.
6. Lagunoff, D., Nicol, D. M., and Pritzl, P. (1973) Lab. Invest. 29, 449-453.
7. Brot, F. E., Glaser, J. H., Roozen, K. J., and Sly, W. S. (1974) Biochem. Biophys. Res. Commun. 57, 1-8.
8. Reisfeld, R. A., Lewis, U. J., and Williams, D. E. (1962) Nature 195, 281-283.
9. Stahl, P. D., and Touster, O. (1971) J. Biol. Chem. 246, 5398-5406.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
11. Musa, B. U., Doe, R. P., and Seal, U. S. (1965) J. Biol. Chem. 240, 2811-2816.
12. Hedrick, J. L., and Smith, A. J. (1968) Arch. Biochem. Biophys. 126, 155-164.
13. Swank, R. T., and Paigen, K. (1973) J. Molec. Biol. 77, 371-389.
14. Dean, R. T. (1974) Biochem. J. 138, 407-413.
15. Potier, M., and Gianetto, R. (1973) Canadian J. Biochem. 51, 973-979.
16. Okochi, T., Masaki, S., Aoki, T., Ito, F., and Yamamura, Y. (1968) Med. J. Osaka Univ. 19, 147-155.
17. Plapp, B. V., and Cole, R. D. (1967) Biochemistry 6, 3676-3681.
18. Higashino, K., Okochi, T., Aoki, T., Ito, F., and Yamamura, Y. (1971) Med. J. Osaka Univ. 21, 251-255.
19. Sakamoto, W., and Nishikaze, O. (1972) Enzyme 13, 211-223.
20. Huddleston, J. F., Lee, G., and Robinson, J. C. (1971) Amer. J. Obstet. Gynec. 109, 1017-1022.
21. Morell, A. G., Gregoriadis, G., Scheinberg, I. H., Hickman, J., and Ashwell, G. (1971) J. Biol. Chem. 246, 1461-1467.
22. Hickman, S., Shapiro, L. J., and Neufeld, E. F. (1974) Biochem. Biophys. Res. Commun. 57, 55-61.