BINDING OF HUMAN LIVER β-GALACTOSIDASES TO PLANT LECTINS INSOLUBILIZED ON AGAROSE

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Received November 8, 1973

SUMMARY: More than 95% of human liver acid β-galactosidases A and B (E.C. 3.2.1.23) are bound by Concanavalin A insolubilized on agarose. A novel feature of acid β-galactosidase elution from Con A-agarose is an optimum in competitor sugar concentration and marked temperature dependence. Wheat-germ agglutinnin binds specifically to 60% of total acid β-galactosidase. Negligible specific binding occurs to L. tetraglobinus L-fucose binding protein. Ganglioside GM_1 β-galactosidase, asialofetuin β-galactosidase and acid 4-methylumbelliferyl β-galactosidase coelute from Con A columns. These enzymes differ from neutral β-galactosidase (β-glucosidase) which is not bound by Con A. These findings suggest the presence of residues resembling α-D-mannose and N-acetyl-D-glucosamine in the acid β-galactosidases.

INTRODUCTION: Previous findings indicate that human liver acid β-galactosidases are sialoproteins (1,2,3). We have explored whether lectins, which bind to sugar residues (4), also bind β-galactosidases; this might provide evidence for the presence of other sugars characteristic of glycoproteins. Since acid β-galactosidase is a marker for lysosomes (5) such a study may be relevant to intralysosomal lectin binding (6). In this report we demonstrate that human liver β-galactosidases which hydrolyze ganglioside GM_1,* asialofetuin (ASF) and 4-methylumbelliferyl β-D-galactoside (4MU β-gal) (8) bind specifically to columns of Concanavalin (Con) A agarose and wheat-germ agglutinin (WGA) agarose.

MATERIALS AND METHODS: The following insolubilized lectins were used: Con A - Sepharose, 8mg/ml resin (Pharmacia); WGA - agarose, 1.45 mg/ml (Miles) and L. tetraglobinus L-fucose binding protein-agarose, 1.68 mg/ml (Miles). Sepharose 4B was from Pharmacia. Methyl α-D-mannoside (Sigma) was recrystallized from 85% v/v n-propanol/water.

Buffer I was 10mM sodium phosphate, 10mM sodium chloride pH 7.00. Human liver obtained at autopsy was used as the source of enzyme which was prepared, unless

*Abbreviations used: GM_1, ganglioside GM_1, nomenclature of Svennerholm (7); ASF, asialofetuin; 4MU, 4-methylumbelliferyl; Con A, Concanavalin A and WGA, wheat-germ agglutinnin. The terms 'Sepharose' and 'Agarose' are used interchangeably.

otherwise stated by method B of (3). All manipulations were at 2-4°C unless otherwise indicated.

Assays of GM_1 β-galactosidase, 4MU β-galactosidase and 4MU β-glucosidase were carried out as previously described (3,8). Asialofetuin was prepared by mild acid hydrolysis of fetuin (Sigma) according to Spiro (9) and tritiated in the terminal galactosyl residues by a modification of the method of Morell et al. (10). The details of the preparation, and a microcolumn assay for hydrolysis of the terminal galactosyl residues from ASF (ASF β-galactosidase) is the subject of a separate report. Under the conditions of assay none of the sugars used as competitors for binding to the lectins inhibits the enzymes more than 5%. Protein was measured by the method of Lowry et al. (11) using a bovine serum albumin standard; a small correction for color depression by methyl-α-D-mannoside was made.

RESULTS AND DISCUSSION: Liver extracts applied to columns of Con A, WGA, L-fucose binding protein and Sepharose 4B, and eluted in both the presence and absence of sugar gave the elution profiles of Fig. 1. A column of ASF covalently bound to Sepharose 4B (1 mg/ml), gave results indistinguishable from unmodified Sepharose 4B. The proportion of activity specifically bound to each lectin is calculated from the difference between total activity eluted in the presence and absence of sugar divided by the total activity applied. Specific binding to each column was as follows: Con A, more than 95%; WGA, 60%; L-fucose binding protein, and Sepharose 4B, less than 5%. Reduction of the volumne of liver supernatant applied to WGA by one half did not significantly change the proportion of specifically bound β-galactosidase. The fraction of β-galactosidase eluted from WGA after application of the sugar was dialyzed against Buffer I (500 vols. 24 hrs.), concentrated by ultrafiltration to one-tenth original volume, and applied to a WGA column; 95% of the activity was specifically bound. The results suggest that there is intrinsic heterogeneity among acid β-galactosidases in binding to WGA. During the course of this wor Bachhawat and Bishayee Subal reported the formation of insoluble complexes of lysosom hydrolases from sheep brain with Con A (12); these are probably analogous findings to those reported here.

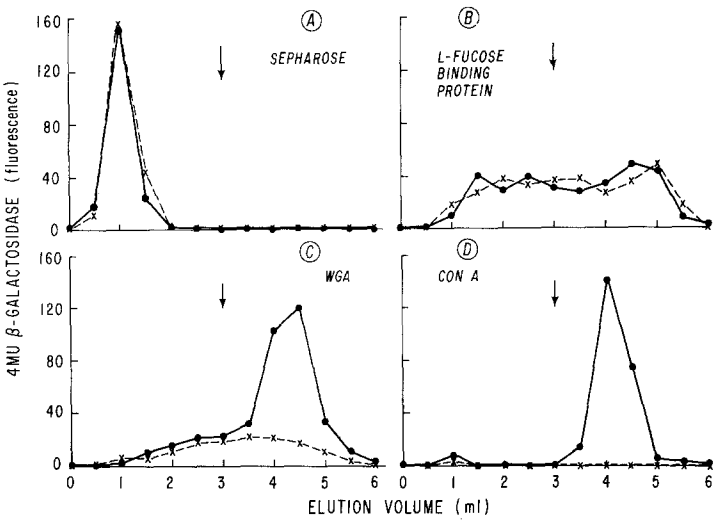

Fig. 1: Chromatography of 4MU β-galactosidase on lectin-agarose columns. Liver supernatant (50µl) prepared as in 'Methods' was applied to 0.4 x 2.0cm lectin-agarose columns at 2°C. They were eluted with 3ml buffer I at the same temperature and a flow rate of 0.1 ml/min. The columns were then shifted to room temperature and elution continued at the same flow rate with buffer I containing the following sugars (●): (a) 0.75M methyl-α-D-mannoside: (b) 0.75M L-fucose; (c) 0.75M N-acetyl-D-glucosamine and (d) 0.75M methyl-α-D-mannoside. Duplicate columns were eluted in the absence of sugar (x). Fraction size was 0.5 ml. 10µl. of a fraction was assayed undiluted with an incubation of 15 min.

Initial attempts at the elution of acid β-galactosidases from Con A were unsuccessful til the temperature dependence of binding was recognized. Almost no activity was eluted methyl-α-D-mannoside at 2°C while quantitative elution occurred at 22°C (Fig. 2(a)). en elution of the activity was examined using different concentrations of methyl-α-D-nnoside (Fig. 2(b)), an optimum concentration for elution was found. This behavior has t been reported previously for a Con A-ligand interaction. No activity was eluted by 1M lium chloride, 1% Triton X-100, 0.5% sodium taurocholate, 0.5% cetylpyridinium chloride, mg/ml dextran (Pharmacia Type 20) dissolved in Buffer I. Neither 50mM sodium acetate 4.50 containing 10mM sodium chloride nor 50mM tris chloride pH 8.50 containing 10mM lium chloride eluted the enzyme. These results indicate that the binding is specifically liated through the interaction of carbohydrate bound to the enzyme protein.

Partially purified β-galactosidases A and B were prepared as described previously (3); :h bound to Con A-agarose and were quantitatively eluted with methyl-α-D-mannoside. Both

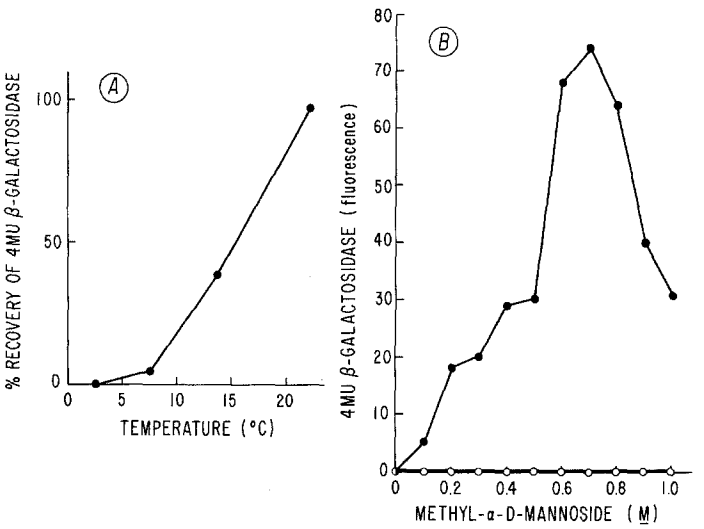

Fig. 2: Elution of 4MU β-galactosidase from Con A-agarose as a function of temperature. Columns were loaded with liver supernatant and eluted with 3ml buffer I at 2°C as described in the legend to Fig. 1. The columns were then shifted to the temperature shown and eluted with 0.75M methyl-α-D-mannoside in Buffer I. Percentage recovery is the total activity in the first 3ml eluted with methyl-α-D-mannoside as a proportion of the total activity applied to the column. (b): Elution of 4MU β-galactosidase from Con A-agarose as a function of methyl-α-D-mannoside concentration at 2°C (o) and 23°C (●). Columns were loaded with liver supernatant and eluted with 3ml. buffer I at 2°C as described in the legend to Fig. 1. They were then shifted to either 23°C or kept at 2°C and eluted with the concentration of methyl-α-D-mannoside shown at the same temperature as the column. Activity of 4MU β-galactosidase shown is the total activity in the first 3ml. eluted after addition of the methyl-α-D-mannoside at the concentration shown. Assay conditions for (a) and (b) are described in the legend to Fig. 1.

forms have been shown previously (3,8) to possess similar pH optima and to cleave 4MU β-galactoside and GM_1, but they differ in molecular weight and electrophoretic mobility. starch gel electrophoresis both were eluted in the same fraction from Con A-agarose with methyl-α-D-mannoside, indicating they both possess the carbohydrate moieties required fo Con A binding.

Neutral β-galactosidase has a pH optimum of 5.5. It cleaves 4MU β-glucopyranoside as well as 4MU β-galactopyranoside but does not hydrolyze GM_1 (3,8). No binding of neutral β-galactosidase to Con A-agarose was observed (Fig. 3).

When activities of GM_1 β-galactosidase, and ASF β-galactosidase were measured in the effluent from Con A-agarose, they corresponded closely to the activity of acid

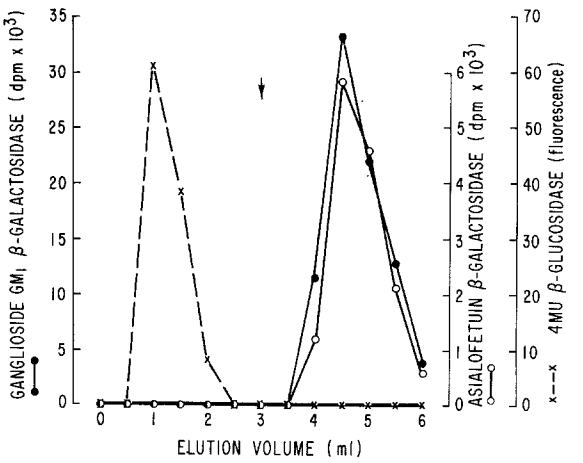

Fig. 3: Chromatography of GM_1 β-galactosidase (•), ASF β-galactosidase (o) and MU β-glucosidase (x) on Con A-agarose. Con A-agarose was loaded with liver supernatant as described in the legend to Fig. 1 except that the volume applied was 0.2ml. lution conditions were as for Fig. 1(d); 0.75M methyl-α-D-mannoside was applied at he point shown by the arrow. To measure 4MU β-glucosidase, 10µl. was incubated in he assay. GM_1 β-galactosidase was assayed on 25µl. of a fraction. ASF β-galactosidase ssays contained 130,000dpm ASF, 0.34 mg/ml ASF and 25µl. of a fraction, 50mM sodium cetate, 5mM sodium chloride pH 4.50; concentrations are final in the assay. The incubation time for all three substrates was 2 hr.

TABLE

Table I

Partial purification of GM_1-and acid 4MU β-galactosidases on Con A-agarose[a]

	Vol ml	Protein mg	GM_1 µmol/min	GM_1 µmol/min/mg	4MU µmol/min	4MU µmol/min/mg
Liver supernatant	8730	123,090	185	0.0015	1220	0.010
Activity eluted with sugar.	390	1,330	158	0.117	1040	0.783

[a]Liver supernatant was prepared by Method A of Ref. 3. Con A Sepharose (5 x 15cm. bed) was loaded with the supernatant at 2°C, washed with 1.5l buffer I at 2°C, shifted to room temperature and eluted with 0.75M methyl-α-D-mannoside in buffer I. The mean flow rate was 3 ml/min during loading and 4 ml/min thereafter.

4MU β-galactosidase (Fig. 3). The large scale partial purification of GM_1 and 4MU β-galactosidase from a high speed liver supernatant is shown in Table 1; an 80-fold purification of the activities was achieved.

These experiments indicate that most molecules of GM_1 β-galactosidase, ASF β-galactosidase and acid 4MU β-galactosidase possess α-D-mannosyl - like residues. Carbohydrate regions containing N-acetyl-D-glucosaminyl - like residues, however, appear to be accessible to WGA on only about 60% of the acid β-galactosidase.

ACKNOWLEDGEMENTS: This project was aided by grants from NIH, Grant NS 08682, USPHS Program Project Grant GM 17702, the National Foundation - March of Dimes and the A.P. Sloan Foundation.

REFERENCES

1. Goldstone, A., Konecny, P. and Koenig, H. (1971). Fed. Eur. Biochem. Soc. Lett. 13, 68-72.

2. Kint, J.A. and Huys, A. (1972), in 'Glycolipids, Glycoproteins and Mucopolysaccharides of the Nervous System' (Zambotti, V., Tettamanti, C. and Arrigoni, M., eds.), p. 273, Plenum Press, New York.

3. Norden, A.G.W. and O'Brien, J.S. (1973). Arch. Biochem. Biophys. 159, 383-392.

4. Lis, H. and Sharon, N. (1973). Annu. Rev. Biochem. 42, 541-574.

5. Barrett, A.J. (1972). in 'Lysosomes' (Dingle, J.T. ed.), p. 79-81, American Elsevier, New York.

6. Henning, R. and Uhlenbruck, G. (1973). Nature (London) New Biol. 242, 120-122.

7. Svennerholm, L. (1963). J. Neurochem. 10, 613-623.

8. Ho, M.W. and O'Brien, J.S. (1971). Clin. Chim. Acta. 32, 443-450.

9. Spiro, R.G. (1960). J. Biol. Chem. 235, 2860-2869.

10. Morrell, A.G., Gregoriadis, G., Scheinberg, I.H., Hickman, J. and Ashwell, G. (1971). J. Biol. Chem. 246, 1461-1467.

11. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). J. Biol. Chem. 193, 265-275.

12. Bachhawat, B.K. and Bishayee Subal. (1973). IXth Intern. Congress of Biochem., Stockholm, Abstract 1el7. Aktiebolaget Egnellska Bokryckeriet, Stockholm.