Vol. 179, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1991 Pages 416-422

GLUCURONOSYL TRANSFER TO GALACTOSE RESIDUES IN THE BIOSYNTHESIS OF HNK-1 ANTIGENS AND XYLOSE-CONTAINING GLYCOSAMINOGLYCANS: ONE OR TWO TRANSFERASES?

Tracy Curenton, Göran Ekborg and Lennart Rodén[1]

Laboratory of Medical Genetics and the Metabolic Diseases Research Laboratory, University of Alabama at Birmingham, Birmingham, Alabama 35294

Received July 17, 1991

Summary. An early step in the assembly of the xylose→serine-linked proteoglycans is the transfer of glucuronic acid to the C-3 position of a galactose residue in the carbohydrate-protein linkage region. Since a similar reaction occurs in the biosynthesis of HNK-1 antigens, the question arose whether these processes are catalyzed by the same enzyme. In the present study, the proteoglycan-related glucuronosyltransferase activity in embryonic chick brain was found to be firmly membrane-associated, while the majority of the activity towards N-acetyllactosamine - a model substrate for HNK-1 antigen biosynthesis - was readily solubilized. No activity towards N-acetyllactosamine was found in embryonic chick cartilage, which is a rich source of the proteoglycan-related enzyme. Together with the results of mixed substrate experiments, these findings strongly indicate the existence of two separate glucuronosyltransferases catalyzing transfer to galactose residues.

One of the early steps in the assembly of the polysaccharide chains of the xylose→serine-linked proteoglycans is the transfer of glucuronic acid from UDP-glucuronic acid to a galactose residue, resulting in the formation of a β1,3 linkage (1). The enzyme catalyzing this reaction - glucuronosyltransferase I - was first detected in embryonic chick cartilage (2) and was subsequently purified partially from embryonic chick brain (3) and a mouse mastocytoma (4). When incubated with a number of potential acceptors containing a nonreducing terminal galactose residue, the crude

[1] To whom correspondence should be addressed at P.O. Box 500, University of Alabama at Birmingham, Birmingham, AL 35294.

Abbreviations used are: Gal-Gal-Xyl-Ser, O-β-D-galactopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→O)-L-serine; Gal-GlcNAc, N-acetyllactosamine; UDP-GlcUA, UDP-D-glucuronic acid; and MOPS, 3-(N-morpholino)propanesulfonic acid.

0006-291X/91 $1.50

enzyme preparations catalyzed transfer not only to proteoglycan fragments of the appropriate structure (e.g., Gal-β1,3-Gal) but also to similar compounds such as lactose (2-4). Notably, Brandt et al. (3) observed in their studies of the chick brain enzyme that N-acetyllactosamine (Gal-β1,4-GlcNAc) was actually a better substrate than Gal-β1,3-Gal, but no particular significance could be attributed to this finding at the time. Recently, however, two glycolipids have been discovered in nerve tissue, which display reactivity towards the HNK-1 antibody and contain a terminal glucuronic acid residue that is sulfated at C-3 and is linked by a β1,3 linkage to the galactose moiety of an N-acetyllactosamine group (5,6). The 3-sulfated glucuronic acid residue has been identified as the epitope of the HNK-1 reactive glycolipids and is probably also present in other HNK-1 antigens residing in glycoproteins and proteoglycans (7-11). Transfer of glucuronic acid to two glycolipids, which may be regarded as the naturally occurring precursors of the HNK-1 reactive compounds, was recently reported by Das et al. (12), using embryonic chick brain as the enzyme source. Somewhat surprisingly, however, transfer to N-acetyllactosamine did not take place under the conditions used in these experiments.

In view of the similarities between the two glucuronosyl transfer reactions described above, we have investigated the problem whether these processes are catalyzed by a single enzyme or whether two separate glucuronosyltransferases are involved. The results described below favor the conclusion that the reactions are catalyzed by two separate enzymes, which, however, exhibit certain similarities in their substrate recognition.

MATERIALS AND METHODS

Gal-Gal-Xyl-Ser was synthesized as previously described (13). MOPS, UDP-glucuronic acid, Triton X-100, and N-acetyllactosamine (Gal-GlcNAc) were purchased from Sigma Chemical Co., St. Louis, MO. Scintiverse E and other chemicals were obtained from Fisher Scientific Co., Atlanta, GA. UDP-[^{14}C]glucuronic acid (328.2 mCi/ mmol) was from NEN Research Products, Boston, MA. Protein was determined by the method of Bradford (14).

Enzyme preparations. Brain (~60 g) from 13-day-old chick embryos was homogenized in an equal volume of 50 mM MOPS, pH 7.0, containing 50 mM KCl and 1 mM EDTA (standard buffer). Total protein in the homogenate was 4.36 g. The homogenate was centrifuged at 100,000 x g for 1 h, the resulting pellet was homogenized in 40 ml of 50 mM MOPS/500 mM KCl/1 mM EDTA/0.025% Triton X-100, pH 7.0, and the mixture was again centrifuged at 100,000 x g for 1 h. The final pellet was resuspended in 40 ml of standard buffer containing

0.025% Triton X-100. The protein contents of the initial 100,000 x g supernatant, the pellet extract, and the final pellet were 1.71, 1.11, and 1.91 g, respectively.

Cartilage from tibiae and femores of 13-day-old chick embryos was homogenized in two volumes of standard buffer, and the homogenate was centrifuged at 10,000 x g for 30 min. The pellet was resuspended in 40 ml of standard buffer.

Enzyme assays. Reaction mixtures had a total volume of 50 µl and, unless otherwise noted, contained 40-90 µg of protein in 25 µl of standard buffer with 0.025% Triton X-100, 5 mM Gal-Gal-Xyl-Ser or Gal-GlcNAc (10 µl of a 25 mM solution in buffer), 50 µM UDP-[^{14}C]GlcUA (0.1 µCi; 10 µl of a 0.5 mM solution in buffer, containing 10 µCi/ml), and 20 mM $MnCl_2$ (5 µl of a 0.2 M solution in buffer). After incubation for 1 h at 37 °C, the reaction was stopped by addition of 50 µl of ethanol, and the mixture was centrifuged in a microfuge at 12,000 rpm for 3 min. A sample (25-50 µl) of the supernatant was applied to Whatman No. 3MM paper (27 x 60 cm), and electrophoresis was carried out at 3000 volts (55 volts/cm) for 0.5-3.0 h in 0.08 M pyridine/0.046 M acetic acid, pH 5.3 (Gilson Model D high voltage electrophorator). After drying, the paper was cut into 1-cm strips, which were soaked in 0.5 ml of water for 1 h, and radioactivity was measured by liquid scintillation spectrometry after addition of 4.5 ml of Scintiverse E.

RESULTS AND DISCUSSION

The cell-free preparations of embryonic chick brain all catalyzed glucuronosyl transfer from UDP-[^{14}C]glucuronic acid to Gal-Gal-Xyl-Ser and N-acetyllactosamine (Gal-GlcNAc). Results of electrophoretic analysis of the reaction mixtures containing the 100,000 x g supernatant fraction of the homogenate and 1.5 mM Gal-GlcNAc or 5 mM Gal-Gal-Xyl-Ser are shown in Fig. 1, from which it is seen that Gal-GlcNAc was the better acceptor and yielded approximately twice as much radioactive product as Gal-Gal-Xyl-Ser.

The results of mixed substrate experiments are shown in Table 1. Upon addition of Gal-GlcNAc, at a final concentration of 1.5 mM, to a reaction mixture containing 5 mM Gal-Gal-Xyl-Ser, a 61% decrease in glucuronosyl transfer to the latter was observed. A similar experiment, with brain homogenate as the enzyme source, resulted in 85% inhibition of transfer to Gal-Gal-Xyl-Ser, when Gal-GlcNAc was present at a final concentration of 5 mM. In contrast, Gal-Gal-Xyl-Ser, at a concentration of 5 or 10 mM, did not significantly affect product formation from Gal-GlcNAc in reaction mixtures containing the latter at a concentration of 1.5 mM. It is thus apparent that glucuronosyltransferase I recognizes Gal-GlcNAc and that, considered by itself, this result is compatible with the assumption that the two transfer reactions are catalyzed by a single enzyme. The lack of a significant effect of Gal-Gal-

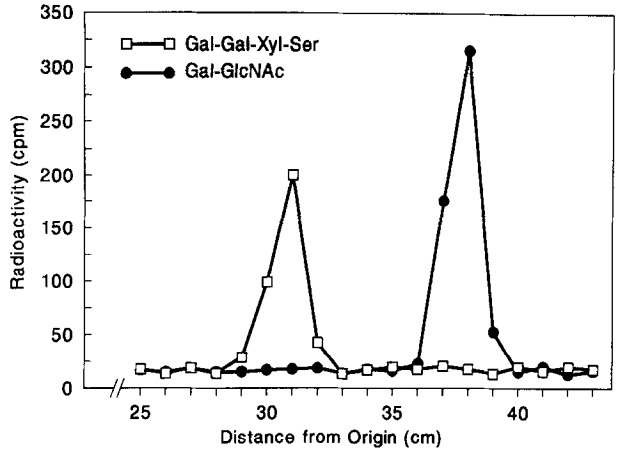

Figure 1. Paper electrophoresis of products of glucuronosyl transfer to Gal-GlcNAc and Gal-Gal-Xyl-Ser. Reaction mixtures contained 1.5 mM Gal-GlcNAc or 5 mM Gal-Gal-Xyl-Ser and the 100,000 x g supernatant fraction (60 μg of protein) of a brain homogenate. Electrophoresis was carried out for 3 h. The product of glucuronosyl transfer to lactose had the same mobility as GlcUA-Gal-GlcNAc (not shown).

Xyl-Ser on transfer to Gal-GlcNAc, however, cannot easily be reconciled with such a conclusion. It should also be recalled that Helting (4) observed transfer to lactose in a crude mastocytoma extract but not with a partially purified preparation of glucuronosyltransferase I.

The existence of two separate glucuronosyltransferases, suggested by the mixed substrate experiments, was further supported by examination of the distribution of the activities towards the

TABLE 1

GLUCURONOSYL TRANSFER TO Gal-Gal-Xyl-Ser AND Gal-GlcNAc IN MIXED SUBSTRATE EXPERIMENTS

Acceptor Concentration (mM)		Radioactivity in Product (cpm)	
Gal-Gal-Xyl-Ser	Gal-GlcNAc	GlcUA-Gal-Gal-Xyl-Ser	GlcUA-Gal-GlcNAc
5		609	
10		1169	
	1.5		1190
5	1.5	240	1024
10	1.5	274	1103

Enzyme source: 100,000 x g supernatant (60 µg of protein).

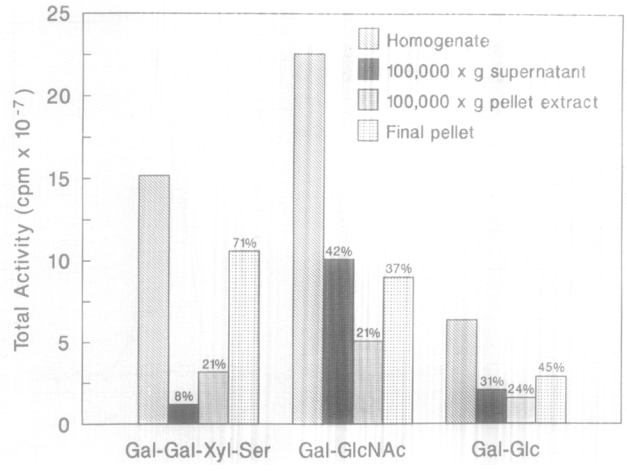

Figure 2. Distribution of glucuronosyltransferase activities in embryonic chick brain fractions. Reaction mixtures contained 5 mM Gal-Gal-Xyl-Ser, N-acetyllactosamine, or lactose and 40-90 μg of protein. Percentages indicated are percent of total recovered activity.

two substrates among the soluble and particulate fractions of the brain homogenate. As shown in Fig. 2, the 100,000 x g supernatant contained 42% of the activity towards Gal-GlcNAc, while only 8% of the activity towards Gal-Gal-Xyl-Ser was found in this fraction. After extraction of the particulate fraction with buffer containing 0.5 M KCl and 0.025% Triton X-100, 71% of the latter activity remained in particulate form, while only 37% of the activity towards Gal-GlcNAc was insoluble under these conditions.

Since lactose (Gal-β1,4-Glc) has been used as a glucuronosyl acceptor in previous studies (2-4) on the assumption that it is a substrate for glucuronosyltransferase I, it was included in the analysis of the distribution of glucuronosyltransferase activities between the various fractions of the brain homogenate. As shown in Fig. 2, the profile obtained with lactose as substrate was similar to that observed for Gal-GlcNAc and was clearly different from that seen with Gal-Gal-Xyl-Ser as the acceptor. These results suggest that lactose is not a substrate for glucuronosyltransferase I and that it may, instead, be an alternative substrate for the enzyme catalyzing transfer to Gal-GlcNAc. This interpretation seems the much more likely, since the only structural difference between lactose and N-acetyllactosamine is that the latter has an N-acetylated amino group, while both the linkage position and the penultimate monosaccharide are different in lactose and the Gal-β1,3-Gal disaccharide unit serving as a substrate for glucuronosyl-

TABLE 2

RATIOS OF PRODUCTS FORMED FROM Gal-Gal-Xyl-Ser, Gal-GlcNAc, AND Gal-Glc IN VARIOUS CHICK BRAIN FRACTIONS

Fraction	Gal-GlcNAc / Gal-Gal-Xyl-Ser	Gal-GlcNAc / Gal-Glc	Gal-Gal-Xyl-Ser / Gal-Glc
Homogenate	1.49	3.50	2.36
100,000 x g supernatant	8.33	4.89	0.59
100,000 x g pellet extract	1.59	3.24	2.04
Final pellet	0.84	3.06	3.61

transferase I. It should also be noted in this context that the K_m values measured for Gal-GlcNAc and Gal-Gal-Xyl-Ser were approximately 1.5 and 5 mM, respectively, and that the conditions of these experiments, therefore, were such that an effect of Gal-Gal-Xyl-Ser on transfer to Gal-GlcNAc should have been observed, if a single enzyme catalyzes transfer to both substrates.

Table 2 shows a quantitative comparison of the three substrates, expressed as ratios of products formed in separate reaction mixtures containing the acceptors at a concentration of 5 mM. It is seen, i.a., that in brain homogenate 1.49 times more product was formed from Gal-GlcNAc than from Gal-Gal-Xyl-Ser, while a ratio of 8.33 was calculated for the 100,000 x g supernatant fraction. In contrast, Gal-Gal-Xyl-Ser was actually the better acceptor, when the final pellet was used as the enzyme source, as indicated by the ratio of 0.84.

Extending the comparison of the relative acceptor activities of Gal-Gal-Xyl-Ser and Gal-GlcNAc to extracts of embryonic chick cartilage, it was found that incubation of the 10,000 x g supernatant (55 µg of protein) and pellet (90 µg of protein) with 5 mM Gal-Gal-Xyl-Ser yielded 986 and 2470 cpm of product, respectively. In contrast, no product was formed from Gal-GlcNAc at a concentration of 5 mM.

Taken together, the findings reported here strongly support the conclusion that glucuronosyl transfer to galactose in the biosynthesis of HNK-1 antigens and xylose-containing glycosaminoglycoglycans is catalyzed by two separate glucuronosyltransferases.

Acknowledgments. This work was supported by NIH grants DE 08252 and NS 27353 and by a Pregraduate Fellowship to T.C. from the March of Dimes Birth Defects Foundation.

REFERENCES

1. Rodén, L. (1980) in The Biochemistry of Glycoproteins and Proteoglycans (Lennarz, W.J., ed) pp. 267-371, Plenum Press, New York

2. Helting, T., and Rodén, L. (1969) J. Biol. Chem. 244, 2799-2805

3. Brandt, A.E., Distler, J., and Jourdian, G.W. (1969) Proc. Natl. Acad. Sci. U.S.A. 64, 374-380

4. Helting, T. (1972) J. Biol. Chem. 247, 4327-4332

5. Chou, D.K.H., Ilyas, A.A., Evans, J.E., Quarles, R.H., and Jungalwala, F.B. (1985) Biochem. Biophys. Res. Commun. 128, 383-388

6. Ariga, T., Kohriyama, T., Freddo, L., Latov, N., Saito, M., Kon, K., Ando, S., Suzuki, M., Hemling, M.E., Rinehart, K.L., Jr., Kusunoki, S., and Yu, R.K. (1987) J. Biol. Chem. 262, 848-853

7. Schwarting, G.A., Jungalwala, F.B., Chou, D.K.H., Boyer, A.M., and Yamamoto, M. (1987) Dev. Biol. 120, 65-76

8. Shashoua, V.E., Daniel, P.F., Moore, M.E., and Jungalwala, F.B. (1986) Biochem. Biophys. Res. Commun. 138, 902-909

9. Margolis, R.K., Ripellino, J.A., Goosen, B., Steinbrich, R., and Margolix, R.U. (1987) Biochem. Biophys. Res. Commun. 145, 1142-1148.

10. Gowda, D.C., Margolis, R.U., and Margolis, R.K. (1989) Biochemistry 28, 4468-4474

11. Hoffman, S., and Edelman, G.M. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 2523-2527

12. Das, K.K., Basu, M., Basu, S., Chou, D.K.H., and Jungalwala, F.B. (1991) J. Biol. Chem. 266, 5238-5243

13. Ekborg, G., Curenton, T., Krishna, N.R., and Rodén, L. (1990) J. Carbohyd. Chem. 9, 15-37

14. Bradford, M. (1976) Anal. Biochem. 72, 248-254