GLYCOSYL TRANSFERASES IN MAMMALIAN GASTRIC MUCOSAL LININGS

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Received August 22, 1967

A proposed mechanism for the biosynthesis of the carbohydrate chains in the blood-group active glycoproteins is through the sequential addition of sugar units to the growing chains in a glycoprotein macromolecule or to low molecular weight carbohydrate intermediate units that are subsequently incorporated into the macromolecules (Watkins and Morgan, 1959; Watkins, 1966). In attempts to test this hypothesis, gastric mucosa, a rich source of blood-group active material, was examined for glycosyl transferases of the appropriate specificity. Low molecular weight compounds of known structure, and glycoproteins, were tested as acceptors. Evidence is presented here for α- and β-galactosyl transferases, and a β-N-acetylglucosaminyl transferase, in particulate preparations from human, baboon and rabbit gastric mucosa, that respectively donate D-galactose and N-acetylglucosamine to low molecular weight acceptors.

Materials and Methods

UDP-galactose-C14 (uniformly labeled; 120 mC/mM) was purchased from the New England Nuclear Corporation. Glucosamine-6-phosphate (Jourdian and Roseman, 1962) labeled in the acetyl group by means of (acetic-1-C14 anhydride, 65 mC/mM) (Distler, Merrick and Roseman, 1958) was converted to UDP-N-acetyl-D-glucosamine-C14 with phosphoglucomutase and UDP-N-acetylglucosamine pyrophosphorylase from bakers' yeast (Brown and Glaser,

1955).

Normal tissue from human stomachs removed by gastrectomy and fresh mucosal linings from baboons and rabbits were homogenised in ice-cold 0.15 M KCl containing 0.05 M mercaptoethanol (2.5 g. tissue to 10 ml. homogenising fluid), filtered through gauze, centrifuged at 0° for 20 min. at 1500 r.p.m., and the supernatant centrifuged at 105,000 g. for 1 hr. at 0°. The deposit was resuspended in KCl-mercaptoethanol and centrifuged again for 1 hr. at 105,000 g. Finally the deposit was resuspended in one-tenth the original volume of KCl-mercaptoethanol. This particulate fraction was used as the enzyme source.

The reaction mixtures used to test for galactosyl and N-acetylglucosaminyl transferases are given in Tables 1 and 2 respectively. After incubation neutral radioactive sugars were separated from the nucleotides and sugar phosphates by paper electrophoresis in 0.2 M ammonium formate buffer pH 3.6, eluted and examined chromatographically (solvent; ethyl acetate:pyridine: water 5:2:2 $\%_v$). The size of the labeled oligosaccharides formed was estimated by reference to compounds of known structure run on the same chromatogram. The anomeric linkage of the galactose-C14 was determined by treatment of aliquots with a β-galactosidase from Trichomonas foetus (Harrap and Watkins, unpublished) and an α-galactosidase from coffee beans (Courtois and Petek, 1966). The liberated galactose-C14 was identified chromatographically. The oligosaccharides containing N-acetylglucosamine-C14 were treated with a β-N-acetylglucosaminidase from T.foetus which was free from α-activity (Harrap and Watkins, unpublished) and the liberated N-acetylglucosamine-C14 was identified by chromatography and by electrophoresis in borate buffer pH 9.5.

Table 1 D-galactosyl transferases in particulate fractions from rabbit stomach mucosal lining

Sugar Acceptor	Size of Saccharide formed	Incorporation of Gal - C14 %	Hydrolysed by Galactosidase	
			α	β
GNAc	Di-	63	-	+
α-Me-GNAc	Di-	40	-	+
β-Me-GNAc	Di-	76	-	+
β-GNAc-(1→4)-GNAc	Tri-	70	-	+
ManNAc	Di-	14	-	+
G	Di-	5	-	+
β-Gal-(1→4)-GNAc	Tri-	33	+	-
α-Fuc-(1→2)-β-Gal-(1→4)-G	Tetra-	33	+	-
α-Fuc-(1→2)-β-Gal-(1→4)-GNAc[1]	Tetra-	25	+	-
α-Fuc-(1→2)-Gal	Tri-	25	+	-
β-Gal-(1→4)-G	Tri-	12	+	-
β-Gal-(1→3)-GNAc	Tri-	6	+	-
Lacto-N-fucopentaose I[2]	Hexa-	5	+	-

Reaction mixture: UDP-Gal-C14, 0.002 μmole (250,000 c.p.m.); ATP, 1 μmole; Tris-HCl pH 7.2, 1.25 μmoles; $MnCl_2$, 0.3 μmole; sugar acceptor 1 μmole; gastric mucosal particle suspension, 25 μl. Total volume 75 μl. Mixtures incubated 16 hr. at 37°. The following sugars did not accept Gal-C14 in this test system (less than 0.1% incorporation); Gal, GalNAc, α-Me-Gal, β-Me-Gal, α-Me-GalNAc, β-Me-GalNAc, Fuc, α-Gal-1-P, N-acetylneuraminic acid, α-Gal-(1→3)-Gal, β-Gal-(1→6)-GNAc, lactodifucotetraose[2], lacto-N-fucopentaose II[2], lacto-N-difucohexaose I[2], lacto-N-difucohexaose II[2]. Abbreviations: GNAc, N-acetyl-D-glucosamine; Gal, D-galactose; ManNAc, N-acetylmannosamine; G, D-glucose; Fuc, L-fucose; GalNAc, N-acetylgalactosamine; Me, methyl; P, phosphate.

Results and Discussion

Incubation of particle suspensions from rabbit tissue with UDP-galactose-C14 and a series of low molecular weight acceptors yielded neutral radioactive compounds of which the main fractions were judged from their chromatographic mobility to be larger than the acceptor by one added sugar residue (Table 1). These labeled

1. Serologically active trisaccharide isolated from human H substance (Rege, Painter, Watkins and Morgan, 1964).
2. Oligosaccharide isolated from human milk (cf. Kuhn, 1957).

products could be divided into two groups according to the anomeric linkage of the galactosyl-C14 residue.

Maximum incorporation of galactose-C14 in β-linkage was observed with acceptors containing terminal non-reducing N-acetyl-β-glucosaminyl residues. The labeled disaccharide formed with N-acetylglucosamine co-chromatographed with β-D-galactosyl-(1→4)-N-acetylglucosamine and was distinguishable from the 3- and 6-position isomers of this disaccharide. Activation of the β-galactosyl transferase by Mn^{++} ions was about ten times as effective as Mg^{++} ions. The enzyme had a broad pH optimum from 6 to 8 and maximum incorporation of D-galactosyl-C14 occurred after incubation for 6 hr. at 37°.

The acceptor sugars for D-galactosyl-C14 in α-linkage contained terminal non-reducing β-D-galactosyl residues either unsubstituted or substituted in the C2 position with L-fucose (Table 1). The α-galactosyl transferase was activated by Mg^{++} and Mn^{++} ions, but Mn^{++} ions were the more effective activators.

The general picture obtained with enzymes from human or baboon stomachs was similar to that given by the rabbit enzymes. Irrespective of the ABO blood groups of the donors, preparations from human and baboon stomachs contained β-galactosyl transferases that added galactose-C14 to N-acetyl-D-glucosamine or β-N-acetyl-D-glucosaminyl-(1→4)-N-acetyl-D-glucosamine. Enzymes catalysing the addition of galactose-C14 in α-linkage to β-D-galactosyl-(1→4)-N-acetylglucosamine and α-L-fucosyl-(1→2)-β-D-galactosyl-(1→4)-glucose were demonstrated in preparations from a human group B stomach and in particle suspensions from several baboon stomachs.

Incorporation of N-acetyl-D-glucosamine-C14 into oligosaccharides occurred with low molecular weight acceptors containing terminal non-reducing β-D-galactosyl residues. With the rabbit

Table 2 N-acetylglucosaminyl transferase in particulate fractions from rabbit stomach mucosal lining

Sugar Acceptor	Size of Saccharide formed	Incorporation of GNAc-C14 %
β-Me-Gal	Di-	12
β-Gal-(1→3)-GalNAc	Tri-	7
β-Gal-(1→4)-GNAc	Tri-	6
β-Gal-(1→3)-GNAc	Tri-	4
β-Gal-(1→6)-GNAc	Tri-	3
β-Gal-(1→3)-G	Tri-	3
β-Gal-(1→4)-G	Tri-	3

Reaction mixture: UDP-GNAc-C14, 0.006 μmole (175,000 c.p.m.); ATP, 2 μmoles: EDTA 2 μmoles; Tris-HCl buffer pH 7.2, 2.5 μmoles; $MnCl_2$ 1 μmole; sugar acceptor 1 μmole; gastric mucosal particle suspension 25 μl. Total volume 95 μl. Mixtures incubated 16 hr. at 37°. All the radioactive oligosaccharides formed were hydrolysed by β-N-acetylglucosaminidase.
The following sugars did not accept GNAc-C14 in this test system (less than 0.1% incorporation): Gal, GNAc, α-Me-Gal, β-GNAc-(1→4)-GNAc, α-Gal-(1→3)-Gal, α-Gal-(1→6)-GNAc, α-Fuc-(1→2)-Gal, α-Fuc-(1→2)-β-Gal-(1→4)-G, lacto-N-fucopentaose II, sucrose, D-mannose, α-nitrophenyl mannoside.
Abbreviations as in Table 1.

enzyme β-methyl galactoside was the best acceptor (Table 2), but with enzymes from human and baboon stomachs β-D-galactosyl-(1→4)-N-acetylglucosamine was a better acceptor than the methyl glycoside. Substitution of the terminal galactosyl residue with L-fucose destroyed the capacity of the sugar to accept N-acetylglucosamine-C14. The N-acetylglucosaminyl transferase is activated by Mn^{++} ions, and slightly by Zn^{++} ions, but activity is not demonstrable in the presence of Mg^{++} ions or Ca^{++} ions. With the rabbit stomach preparation maximum incorporation of N-acetylglucosamine-C14 into the disaccharide formed with β-methyl galactoside occurred at pH 7 in 4 hr.

The significance of these transferases in relation to the

biosynthesis of blood-group substances must await further work, but their specificity with regard to the nature and anomeric linkage of the sugar in the acceptor molecule is consistent with that required to build up some of the units known to occur in the carbohydrate chains of the blood-group active glycoproteins (cf. Watkins, 1966).

This work was supported by a grant from the Medical Research Council.

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