An α -N-Acetyl-D-galactosaminyltransferase Associated with the Human Blood-Group A Character

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(Received 17 June 1968)

The step in the biosynthesis of blood-group A specific structures that is under the control of the A gene is postulated as the addition of N-acetyl-D-galactosamine (2-acetamido-2-deoxy-D-galactose) in α -(1 $\rightarrow$ 3)-linkage to an H-specific structure (Watkins, 1958, 1967; Watkins & Morgan, 1959). The enzymic product of the A gene is thus envisaged as an α -N-acetyl-D-galactosaminyltransferase. Evidence for such an enzyme in stomach mucosal lining from group A baboons (cited in Race, Zideman & Watkins, 1968) and in milk from women of groups A and AB (Kobata, Grollman & Ginsburg, 1968) has been given. An α -N-acetyl-D-galactosaminyltransferase occurring in preparations from human submaxillary glands from group A and AB donors and absent from the glands of blood-group O and B donors is described in the present paper.

The human submaxillary glands were post-mortem specimens removed 20–36 hr. after death. The glands were stored at -18° for 18–20 hr. and then thawed, and the tissue was homogenized in a mechanically driven Potter homogenizer for 4–5 min. in ice-cold 0.15 M-KCl containing 0.05 M-2-mercaptoethanol (5 g. of tissue to 10 ml. of homogenizing fluid). The homogenate was filtered through gauze and centrifuged at 0° for 20 min. at 1100g, and the supernatant was centrifuged at 105 000g for 1 hr. at 0° in a Spinco preparative ultracentrifuge. The deposit from 5 g. of tissue was washed once in KCl-mercaptoethanol, centrifuged for 1 hr. at 105 000g and finally resuspended in 0.5 ml. of the same solution. This particulate preparation was used as the enzyme source. UDP-N-acetyl-D-[1- 14 C]galactosamine was purchased from the International Chemical and Nuclear Corp., City of Industry, Calif., U.S.A., or prepared from D-[1- 14 C]galactosamine (obtained from The Radiochemical Centre, Amersham, Bucks.) by a modification of the procedure of Carlson, Swanson & Roseman (1964).

The reaction mixture used to test for the N-acetylgalactosaminyltransferase is given in Table 1. At the end of the incubation time the neutral sugars were separated from unchanged UDP-N-acetyl-[14 C]galactosamine and N-acetyl-[14 C]galactosamine 1-phosphate by paper electrophoresis in 0.2 M-ammonium formate buffer, pH 3.6, eluted and

chromatographed on Whatman no. 40 paper in solvent *a* (ethyl acetate-pyridine-water, 2:1:2, by vol.). When 2'-fucosyl-lactose [*O*- α -L-fucosyl-(1 $\rightarrow$ 2)-*O*- β -D-galactosyl-(1 $\rightarrow$ 4)-D-glucose] was used as acceptor, transfer of radioactivity from UDP-N-acetyl-[14 C]galactosamine was observed with all the particulate preparations obtained from group A₁ donors, but there was no transfer of radioactivity to this acceptor when enzyme preparations from group O or B donors were tested. The amount of incorporation varied considerably with glands from different group A₁ donors, but this was probably related to the length of time, and other conditions, obtaining between death and removal of the gland. The largest incorporation was observed with a gland from a group A₁B donor (no. 26, Table 1). This gland was removed 21 hr. after death, and the α -D-galactosyltransferase associated with the blood-group B character (Race *et al.* 1968) was also very active. Similarly the group B gland (no. 31), which failed to transfer N-acetyl-[14 C]galactosamine to 2'-fucosyl-lactose, nevertheless transferred to this acceptor [14 C]galactose from UDP-[14 C]galactose. The N-acetylgalactosaminyltransferase activity was demonstrated in particulate preparations from the glands of both secretors and non-secretors (cf. Race & Sanger, 1962) of group A₁. This observation is in accordance with the proposal that the secretor gene controls the biosynthesis of the H-active structures that constitute the substrate for the product of the A or B genes and does not directly influence the expression of these genes (Watkins, 1964).

Other fucose-containing oligosaccharides were tested as acceptors of N-acetyl-[14 C]galactosamine (Table 1). Two compounds containing an α -fucosyl residue linked (1 $\rightarrow$ 2) to galactose, namely the disaccharide, 2'-fucosylgalactose [*O*- α -L-fucosyl-(1 $\rightarrow$ 2)-galactose] and the pentasaccharide, lacto-N-fucopentaose I (Kuhn, Baer & Gauhe, 1956), were good acceptors when a preparation from a group A₁ donor was used as the enzyme source, whereas lacto-N-fucopentaose II (Kuhn, Baer & Gauhe, 1958), in which the L-fucose residue is on the subterminal N-acetylglucosamine, and lacto-difucotetraose (Kuhn & Gauhe, 1958), which is substituted with two fucose residues on adjacent sugars, were poor acceptors in the same system.

Table 1. *Transfer of N-acetyl-D-[¹⁴C]galactosamine from UDP-N-acetyl-D-[¹⁴C]galactosamine to fucose-containing acceptors by enzyme preparations from human submaxillary glands*

The reaction mixtures contained: UDP-N-acetyl-D-[¹⁴C]galactosamine, 0.13 μ mole (200 000 counts/min.); tris-HCl buffer, pH 7.2, 1.25 μ moles; MnCl₂, 0.8 μ mole; sugar acceptor, 1 μ mole; ATP, 1.5 μ moles; enzyme particle suspension, 25 μ l. The total volume was 108 μ l. The mixtures were incubated for 17 hr. at 37°. The R_{lactose} values were obtained with solvent α (see the text). Radioactivity was counted on a Packard radiochromatogram scanner and absence of detectable radioactivity is indicated by 0.

Submaxillary gland no.	Blood group	Secretor status	Acceptor sugar	R_{lactose} of product	Incorporation of N-acetyl-D-[¹⁴ C]galactosamine	
					(counts/min.)	(% of total recovered activity)
16	A ₁	Secretor	2'-Fucosylgalactose	1.0	29 500	23
			2'-Fucosyl-lactose	0.5	29 200	22
18	A ₁	Secretor	2'-Fucosyl-lactose	0.5	36 000	36
			Lacto-N-fucopentaose I	0.2	21 000	21
			Lacto-N-fucopentaose II	0.13	1 700	2
			Lactodifucotetraose	0.27	1 600	2
19	A ₁	Secretor	2'-Fucosyl-lactose	0.50	6 600	6
20	A ₁	Non-secretor	2'-Fucosyl-lactose	0.50	7 800	10
23	A ₁	Non-secretor	2'-Fucosylgalactose	1.0	2 900	3
			2'-Fucosyl-lactose	0.50	4 700	5
26	A ₁ B	Secretor	2'-Fucosylgalactose	1.0	117 000	46
			2'-Fucosyl-lactose	0.50	135 000	53
			Lacto-N-fucopentaose I	0.2	99 000	39
15	O	Secretor	2'-Fucosylgalactose	—	0	—
			2'-Fucosyl-lactose	—	0	—
17	O	Secretor	2'-Fucosyl-lactose	—	0	—
			Lacto-N-fucopentaose I	—	0	—
			Lacto-N-fucopentaose II	—	0	—
			Lactodifucotetraose	—	0	—
31	B	Secretor	2'-Fucosylgalactose	—	0	—
			2'-Fucosyl-lactose	—	0	—

The disaccharides 3-O- β -D- and 4-O- β -D-galactosyl-N-acetylglucosamine did not accept N-acetyl[¹⁴C]-galactosamine, indicating that substitution of the galactosyl residue with L-fucose is a substrate requirement of the transferase.

To determine the anomeric linkage of the transferred sugar, and to ascertain that epimerization to N-acetylglucosamine had not taken place, the labelled oligosaccharides formed with O- α -L-fucosyl-(1 $\rightarrow$ 2)-galactose, 2'-fucosyl-lactose and lacto-N-fucopentaose I were treated with an enzyme preparation from *Trichomonas foetus* that destroys blood-group A activity with the release of N-acetylglactosamine (Harrap & Watkins, 1964, and unpublished work). This enzyme, which hydrolyses methyl α -N-acetylglactosaminide but not methyl β -N-acetylglactosaminide, released all the radioactivity from the labelled oligosaccharides. The liberated radioactive sugar was identified as

N-acetylglactosamine by chromatography on borate-impregnated paper in butan-1-ol-pyridine-water (6:4:3, by vol.) (Cardini & Leloir, 1957). The incorporated sugar was therefore N-acetylglactosamine joined to the acceptor substrate in α -linkage.

Hydrolysis of the radioactive trisaccharide formed with O- α -L-fucosyl-(1 $\rightarrow$ 2)-galactose under conditions selected to give a preferential release of L-fucose (2N-acetic acid for 18 hr. at 100°) yielded a product, with R_{lactose} 1.2 in solvent α , which co-chromatographed with the A-active disaccharide, N-acetyl-O- α -D-galactosaminyl-(1 $\rightarrow$ 3)-galactose (Côté & Morgan, 1956; Schiffman, Leskowitz & Kabat, 1962).

The pathway proposed for the formation of an A-specific structure (cf. Watkins, 1958, 1967) is shown in Scheme 1. The α -N-acetylglactosaminyl-transferase present in submaxillary glands from human group A donors, and absent from group O or

Scheme 1. Proposed pathway for the formation of an A-specific structure. Abbreviations: Gal, galactose; GalNAc, N-acetylgalactosamine; Fuc, fucose; R represents the remainder of the molecule.

B donors, which is described in this paper, therefore fulfils many of the requirements postulated for the enzymic product of the blood-group A gene.

We thank Dr Adeline Gauhe for the oligosaccharides from human milk, and Professor F. E. Camps and Dr S. Leibowitz for the submaxillary-gland specimens. This work was supported by a grant from the Medical Research Council.

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