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

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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→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, Ziderman & 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 20–36hr. after death. The glands were stored at −18° for 18–20hr. and then thawed, and the tissue was homogenized in a mechanically driven Potter homogenizer for 4–5min. in ice-cold 0·15M-KCl containing 0·05M-2-mercaptoethanol (5g. of tissue to 10ml. of homogenizing fluid). The homogenate was filtered through gauze and centrifuged at 0° for 20min. at 1100*g*, and the supernatant was centrifuged at 105000*g* for 1hr. at 0° in a Spinco preparative ultracentrifuge. The deposit from 5g. of tissue was washed once in KCl–mercaptoethanol, centrifuged for 1hr. at 105000*g* and finally resuspended in 0·5ml. 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 0·2M-ammonium formate buffer, pH3·6, 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→2)-*O*-β-D-galactosyl-(1→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 A_1 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 A_1 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 A_1B donor (no. 26, Table 1). This gland was removed 21hr. 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 A_1. 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→2) to galactose, namely the disaccharide, 2′-fucosylgalactose [*O*-α-L-fucosyl-(1→2)-galactose] and the pentasaccharide, lacto-*N*-fucopentaose I (Kuhn, Baer & Gauhe, 1956), were good acceptors when a preparation from a group A_1 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-[^{14}C]*galactosamine from UDP*-N-*acetyl*-D-[^{14}C]*galactosamine to fucose-containing acceptors by enzyme preparations from human submaxillary glands*

The reaction mixtures contained: UDP-*N*-acetyl-D-[^{14}C]galactosamine, 0·13 μmole (200000 counts/min.); tris–HCl buffer, pH 7·2, 1·25 μmoles; $MnCl_2$, 0·8 μmole; sugar acceptor, 1 μmole; ATP, 1·5 μmoles; enzyme particle suspension, 25 μl. The total volume was 108 μl. The mixtures were incubated for 17 hr. at 37°. The $R_{lactose}$ values were obtained with solvent *a* (see the text). Radioactivity was counted on a Packard radiochromatogram scanner and absence of detectable radioactivity is indicated by 0.

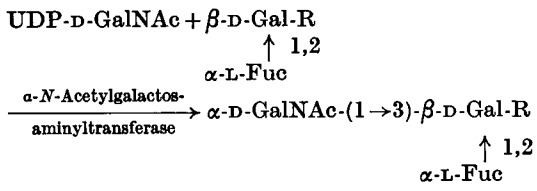
Submaxillary gland no.	Blood group	Secretor status	Acceptor sugar	$R_{lactose}$ of product	Incorporation of *N*-acetyl-D-[^{14}C]galactosamine (counts/min.)	Incorporation of *N*-acetyl-D-[^{14}C]galactosamine (% of total recovered activity)
16	A_1	Secretor	2′-Fucosylgalactose	1·0	29500	23
			2′-Fucosyl-lactose	0·5	29200	22
18	A_1	Secretor	2′-Fucosyl-lactose	0·5	36000	36
			Lacto-*N*-fucopentaose I	0·2	21000	21
			Lacto-*N*-fucopentaose II	0·13	1700	2
			Lactodifucotetraose	0·27	1600	2
19	A_1	Secretor	2′-Fucosyl-lactose	0·50	6600	6
20	A_1	Non-secretor	2′-Fucosyl-lactose	0·50	7800	10
23	A_1	Non-secretor	2′-Fucosylgalactose	1·0	2900	3
			2′-Fucosyl-lactose	0·50	4700	5
26	A_1B	Secretor	2′-Fucosylgalactose	1·0	117000	46
			2′-Fucosyl-lactose	0·50	135000	53
			Lacto-*N*-fucopentaose I	0·2	99000	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[^{14}C]-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 → 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*-acetylgalactosamine (Harrap & Watkins, 1964, and unpublished work). This enzyme, which hydrolyses methyl α-*N*-acetylgalactosaminide but not methyl β-*N*-acetylgalactosaminide, released all the radioactivity from the labelled oligosaccharides. The liberated radioactive sugar was identified as *N*-acetylgalactosamine 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*-acetylgalactosamine joined to the acceptor substrate in α-linkage.

Hydrolysis of the radioactive trisaccharide formed with *O*-α-L-fucosyl-(1 → 2)-galactose under conditions selected to give a preferential release of L-fucose (2 N-acetic acid for 18 hr. at 100°) yielded a product, with $R_{lactose}$ 1·2 in solvent *a*, which co-chromatographed with the A-active disaccharide, *N*-acetyl-*O*-α-D-galactosaminyl-(1 → 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*-acetylgalactosaminyltransferase 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.

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