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An α-D-Galactosyltransferase Associated with the Blood-Group B Character

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The blood-group substances in human secretions with immunological specificities related to the A, B, H and Le^a antigens on the erythrocyte surface are glycoproteins having a major carbohydrate component. Blood-group specificity is associated with the nature, sequence and linkage of the sugar residues at the non-reducing ends of the oligosaccharide chains in the glycoproteins (cf. Morgan, 1965; Watkins, 1966). The release of *N*-acetyl-D-galactosamine from A substance and of D-galactose from B substance leads respectively to the loss of the corresponding A and B serological activities and to the exposure in both substances of H specific structures. This evidence, together with other biochemical, serological and genetical data, led to the formulation of schemes for the biosynthesis of blood-group substances in which the role of the blood-group genes was envisaged as the production of enzymes that control the 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, 1958, 1967; Watkins & Morgan, 1959). On the basis of these predictions the primary products of the *A* and *B* genes are α-*N*-acetyl-D-galactosaminyl- and α-D-galactosyl-transferases respectively that require the presence of the non-reducing end structure of an H-active grouping, namely α-L-fucosyl-(1→2)-D-galactose (Rege, Painter, Watkins & Morgan, 1964), as an acceptor substrate.

The erythrocytes from all rabbits absorb β-agglutinins from human anti-B sera (cf. Wiener, 1943) and autolysates of rabbit stomach tissue have group B activity. Preliminary experiments to test for an enzyme with the characteristics of the product of the blood-group *B* gene were therefore carried out on stomach mucosal linings from rabbits (Ziderman, Gompertz, Smith & Watkins, 1967). A transferase that conveyed an α-D-[^{14}C]galactosyl residue from UDP-D-[^{14}C]galactose to acceptors containing β-linked D-galactose residues was demonstrated. When the terminal galactosyl residue was substituted in the 2-position with α-L-fucose good incorporation of radioactivity was obtained, whereas with compounds such as lacto-*N*-fucopentaose II (Kuhn, Baer & Gauhe, 1958), in which the L-fucose residue is on the subterminal *N*-acetylglucosamine, or compounds substituted with two fucose residues on adjacent sugars, such as lactodifucotetraose (Kuhn & Gauhe, 1958) or lacto-*N*-difucohexaose I (Kuhn & Gauhe, 1960), no incorporation was observed. Preliminary examination of human and baboon stomach mucosal linings revealed a similar α - D - galactosyltransferase (Ziderman *et al.* 1967). Evidence is now presented that this α-D-galactosyltransferase occurs only in the tissues of group B donors and that the products formed have the chemical characteristics expected of B-active structures.

The most potent normal sources of secreted blood-group glycoproteins are gastric mucosa and saliva (cf. Kabat, 1956). Stomach mucosal linings and submaxillary glands were therefore examined as sources of enzymes involved in the biosynthesis of the specific substances. The ABO blood groups of the baboons were determined on the basis of the A, B or H substances secreted in their saliva and on the presence of anti-A or anti-B agglutinins in their serum (Moor-Jankowski, Wiener & Gordon, 1964). The stomachs and submaxillary glands were removed from the baboons immediately after death and the tissues used on the same day. The human submaxillary glands were post-mortem specimens that were frozen for 18hr. before the experiments were carried out. The preparation of the particulate fractions of stomach mucosal linings or submaxillary glands used as enzyme source and the subsequent separation of the neutral ^{14}C-labelled sugars from the other components of the reaction mixture were carried out as described by Ziderman *et al.* (1967). Fucosyl - lactose [α - L - fucosyl - (1 → 2) - β - D - galactosyl-(1→4)-D-glucose], α-L-fucosyl-(1→2)-D-galactose and lactosamine [β-D-galactosyl-(1→4)-*N*-acetyl-D-glucosamine] were tested as acceptor substrates. The results obtained with fucosyl-lactose are given in Table 1. Incorporation of radioactivity into a compound with the chromatographic mobility of a tetrasaccharide [$R_{lactose}$ 0·25 in ethyl acetate–pyridine–water (5:2:5, by vol.) (solvent *a*)] occurred with all the tissues obtained from group B donors, whereas negligible radioactivity was detected in the same area when the enzyme preparations came from donors belonging to group A or O. The labelled tetrasaccharide was not hydrolysed by a purified β-D-galactosidase enzyme from *Trichomonas foetus* (G. J. Harrap & W. M. Watkins, unpublished work), but complete

Table 1. *Transfer of* D-[^{14}C]*galactose from UDP-*D-[^{14}C]*galactose to fucosyl-lactose by enzyme preparations from human and baboon tissues*

The reaction mixtures contained: UDP-D-[^{14}C]galactose, 2 mμmoles (150000 counts/min.); ATP, 2 μmoles; tris–HCl buffer, pH 7·2, 1·25 μmoles; $MnCl_2$, 0·8 μmole; fucosyl-lactose, 1 μmole; enzyme-particle suspension, 25 μl. The total volume was 95 μl. The mixtures were incubated for 16 hr. at 37°. Radioactivity was counted on a Packard Radiochromatogram Scanner, and the absence of detectable radioactivity is indicated by 0.

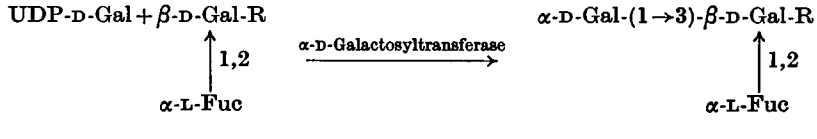
Species	Blood group	Enzyme source	Incorporation of [^{14}C]galactose (counts/min.)	Incorporation of [^{14}C]galactose (% of total recovered radioactivity)
Baboon no. 393	B	Stomach	10000	9
Baboon no. 404	B	Stomach	7800	6
Baboon no. 423	B	Stomach	5800	5
Baboon no. 424	B	Stomach	19000	13
Baboon no. 433	B	Stomach	8500	7
		Submaxillary gland	3000	3
Baboon no. 439	B	Stomach	1700	2
		Submaxillary gland	1700	2
Baboon no. 414	AB	Stomach	5000	5
Baboon no. 394	A	Stomach	0	—
Baboon no. 434	A	Stomach	0	—
Baboon no. 438	A	Stomach	0	—
		Submaxillary gland	0	—
Baboon no. 413	O	Stomach	0	—
Human no. 3	B	Submaxillary gland	18000	14
Human no. 1	A	Submaxillary gland	0	—
Human no. 4	A	Submaxillary gland	0	—
Human no. 5	O	Submaxillary gland	0	—

release of the radioactive sugar was obtained when coffee-bean α-galactosidase (cf. Courtois & Petek, 1966) or the B-destroying enzyme from *T. foetus* (Watkins, 1956) was used as the source of hydrolytic enzyme. The liberated sugar was identified chromatographically as [^{14}C]galactose in solvent *a*.

The disaccharide α-L-fucosyl-(1→2)-D-galactose was also a good acceptor of an α-D-[^{14}C]galactosyl residue when tissues from group B donors were used as the enzyme source, and the amount of radioactivity incorporated was comparable with that found with fucosyl-lactose. The labelled trisaccharide formed had $R_{lactose}$ 0·71 in solvent *a*. Acid hydrolysis of the trisaccharide under conditions that gave preferential release of L-fucose from fucosyl-lactose (N-acetic acid for 4 hr. at 100°) yielded a radioactive disaccharide that co-chromatographed with the B-active disaccharide (Painter, Watkins & Morgan, 1962), α-D-galactosyl-(1→3)-D-galactose [$R_{lactose}$ 1·0 in ethylacetate–pyridine–water (10:4:3, by vol.)], and was clearly distinguishable from α-(1→6)-linked galactobiose. Further work is required to prove that the linkage in the synthesized compound is (1→3), but the results so far obtained do not disagree with this interpretation.

Particulate preparations from rabbit stomach mucosal linings transfer an α-D-[^{14}C]galactosyl residue from UDP-[^{14}C]galactose to lactosamine to form a trisaccharide ($R_{lactose}$ 0·57 in solvent *a*). Preliminary experiments indicated that lactosamine was also an acceptor for the α-D-[^{14}C]galactosyl residues transferred by the human and baboon enzyme preparations (Ziderman *et al.* 1967), but this is now shown to be incorrect because in the present series of experiments none of the enzyme preparations from group B donors gave more than a trace of incorporation when lactosamine was used as an acceptor substrate. Substitution of the terminal β-D-galactosyl unit in the acceptor substrate with an α-L-fucose residue therefore appears to be a necessary requirement for α-D-galactosyltransferase to catalyse the addition of the D-galactose unit.

The pathway proposed for the formation of a B-specific structure (cf. Watkins, 1967) is:



where Fuc represents a fucosyl residue and R represents a hydroxyl group or sugar residue. The distribution and specificity of the galactosyltransferase described in this paper fulfils many of the requirements postulated for the enzyme product of the *B* gene. Thus the enzyme: (1) occurs only in the tissues of group B or AB donors, and is not demonstrable in tissues from donors belonging to groups A and O; (2) transfers an α-D-galactosyl residue; (3) requires an acceptor containing a terminal β-D-galactosyl unit substituted with L-fucose; (4) most probably transfers the α-D-galactosyl unit to C-3 of the β-D-galactosyl residue in the acceptor. Tissues from group A donors would be expected to have an α-*N*-acetyl-D-galactosaminyltransferase with similar properties. The existence of this enzyme is indicated from an experiment in which UDP - *N* - acetyl - D - [^{14}C] - galactosamine was used as sugar donor in place of UDP-D-[^{14}C]galactose; addition of *N*-acetyl-D-[^{14}C]galactosamine to α - L - fucosyl - (1 → 2) - D - galactose (5000 counts/min.) occurred when stomach tissue from group A baboon no. 438 (cf. Table 1) was used as the enzyme source, whereas no addition of labelled hexosamine was observed with the enzyme preparation from group B baboon no. 433 (V. M. Hearn & W. M. Watkins, unpublished work). The exact sequence of events by which the macromolecular blood-group-active glycoproteins are synthesized still remains to be established. The correlation of specific enzymes with certain blood-group characters nevertheless provides a valuable tool for the further investigation of this problem and indicates the validity of the earlier proposals for the functions of the blood-group genes.

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