α-L-FUCOSYLTRANSFERASES IN HUMAN SUBMAXILLARY GLAND AND STOMACH TISSUES ASSOCIATED WITH THE H, Le^a AND Le^b BLOOD-GROUP CHARACTERS AND ABH SECRETOR STATUS

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Schemes for the biosynthetic steps in the formation of the blood group active glycoproteins in secretions postulate that α-L-fucosyltransferases specified by the H and Le genes control the addition of L-fucose to different acceptor sites in a precursor substance to give H, Le^a and Le^b serologically active structures. [1,2]. The presence of 2-, 3- and 4-α-L-fucosyltransferases in submaxillary glands and stomach mucosal linings from persons who are secretors of ABH, and of 3- and 4-α-L-fucosyltransferases in tissues from non-secretor persons, is described in this paper.

Materials and Methods. GDP-D-[U-^{14}C]-mannose (136 mCi/mM; New England Nuclear Corporation) was converted to GDP-L-[^{14}C]-fucose with an enzyme preparation from Aerobacter aerogenes. [3]. Human submaxillary glands removed at post mortem 24-48 hours after death, were chilled and used within 24 hours. The stomachs were gastrectomy specimens, chilled immediately after the operation, and used within 24 hours. Particulate preparations were prepared from the glands or stomach mucosa as described previously. [4].

Abbreviations: GDP, Guanosine diphosphate; Gal, D-galactose; GNAc, N-acetyl-D-glucosamine; Fuc, L-fucose; Glc, D-glucose; Tal, D-talose

The particles from 1 g. of tissue were resuspended in either 0.1 ml of 0.15 M KCl containing 0.05 M mercaptoethanol (Method 1) or in 0.1 ml of the supernatant obtained from the first centrifugation at 105,000 g (Method 2).

The reaction mixture used to test for fucosyltransferases is given in Table 1. Neutral radioactive sugars, separated from the charged components of the reaction mixture by paper electro-

Table 1. α-L-Fucosyltransferases in Human Submaxillary Glands

Reaction Mixture: GDP-L-^{14}C fucose, 0.67 mμmoles; Tris-HCl buffer, pH 7.2, 1.0 μmole; $MgCl_2$, 0.25 μmoles; sugar acceptor, 1 μmole; GTP, 1 μmole; enzyme particle suspension 25 μl. Total volume 105 μl. Mixtures incubated 15 hours at 37°.

Gland No	Blood Group	Secretor Status	Acceptor	Probable Identity of Product	Radioactivity c.p.m.	Radioactivity % total recovered counts
63 *	A	Non-sec	LNB1	4-FucLNB1	29,000	28
			LNAc	3-FucLNAc	42,000	50
			Lact	3-FucLact	20,000	20
40 *	A	Sec	LNB1	2'-FucLNB1	11,000	12
				4-FucLNB1	4,400	5
				2'4-FucLNB1	1,200	1
			LNAc	2'-FucLNAc	6,900	7
				3-FucLNAc	5,400	5
20 *	A	Sec	LNB1	2'-FucLNB1	10,000	9
				4-FucLNB1	59,000	50
			LNAc	2'-FucLNAc	57,000	43
				3-FucLNAc	51,000	38
			Lact	2'-FucLact	7,200	7
				3-FucLact	28,000	25
147 *	O	Sec	LNB1	2'-FucLNB1	4,100	8
				4-FucLNB1	6,200	12
				2'4-FucLNB1	1,500	4
			LNAc	2'-FucLNAc	7,900	15
				3-FucLNAc	900	2
				2'3-FucLNAc	660	1

* Particle suspension prepared by Method 1
* Particle suspension prepared by Method 2
Abbreviations: LNB1, Lacto-N-biose 1; LNAc, N-acetyllactosamine; Lact, Lactose; 2'-FucLNB1, α-Fuc-(1→2)-β-Gal-(1→3)-GNAc; 4-FucLNB1, β-Gal-(1→3)- α-Fuc-(1→4)- GNAc; 2'4-FucLNB1, α-Fuc-(1 2)-β-Gal-(1 3)- α-Fuc-(1→4)- GNAc; 2'-FucLNAc, α-Fuc-(1→2)-β-Gal-(1→4)-GNAc; 3 -FucLNAc, β-Gal-(1→4)- α-Fuc-(1→3)- GNAc; 2'3-FucLNAc, α-Fuc-(1→2)-β-Gal-(1→4)- α-Fuc-(1→3)- GNAc; 2'-FucLact, α-Fuc-(1→2)-β-Gal-(1→4)-Glc; 3-FucLact, β-Gal-(1→4)- α-Fuc-(1→3)- Glc; 2'3-FucLact, Lactodifucotetraose.

phoresis in 0.2 M ammonium formate buffer pH 3.6, were eluted and examined chromatographically on Whatman No. 40 paper (solvent a ethyl acetate-pyridine-water, 10:4:3 v/v; solvent b, ethyl acetate-pyridine-water, 2:1:2 v/v). The electrophoresis strips and chromatograms were counted for radioactivity on a Packard Radiochromatogram Scanner. The anomeric form and position of the fucosyl linkages were determined by treatment of the radioactive oligosaccharides with α-L-fucosidases from Trichomonas foetus. [2]. An extract of the organisms was fractionated on a column of Sephadex G200 and the fractions tested for their action on 2'-fucosyllactose, lacto-difucotetraose and lacto-N-fucopentaose II[‡]. Two fractions were selected; Prep. 1 released L-fucose joined in α(1→2) linkage to galactose but not fucose joined in α(1→3) linkage to glucose or α(1→4) linkage to N-acetyl-glucosamine; Prep. 2 hydrolysed fucose joined in α(1→2), α(1→3) and α(1→4) linkages. The presence of α(1→2) linkages to galactose was also established by alkaline degradation.[6] A solution of the radioactive oligosaccharide (20μl) was mixed with 5 μl of 2'-fucosyl-lactose (2%) and heated for 20 minutes at 75° in a sealed tube with 20 μl of 10% triethylamine in 50% aqueous methanol. The products were examined by chromatography in solvents a and b and the $R_{Lactose}$ (R_L) values of the radioactive peaks compared with those given by authentic samples of α-Fuc-(1→2)-Gal and L-fucose. Under the alkaline conditions selected only very small quantities of α-Fuc-(1→2)-Tal were formed.

Results and Discussion. Submaxillary glands from eleven donors (five group A and four group O secretors, and two A non-secretors) and three stomach specimens (one A, one B and one O secretor) were examined for fucosyltransferases. A number of different

‡. See Reference 5 for the structures of these compounds.

sugars of low molecular weight were tested as acceptors. Galactose was not an acceptor, and the tetrasaccharides, lacto-N-tetraose [5] and lacto-N-neotetraose,[7] although good acceptors of labelled fucose, yielded a mixture of radioactive products that were not readily characterised. The most clear cut results were obtained with lacto-N-biose 1 (β-Gal-(1→3)-GNAc), N-acetyllactosamine (β-Gal-(1→4)-GNAc) and lactose (β-Gal-(1→4)-Glc), all of which were acceptors of L-[^{14}C]-fucose (Tables 1 and 2). When preparations from gland No. 40 or stomach No. 45 were used as the enzyme source six radioactive peaks were detected on the chromatogram of the neutral sugars isolated from incubation mixtures in which lacto-N-biose 1 was used as the acceptor. Peak 1 represented material that stayed on the origin of the chromatogram and peak 6 corresponded to L-fucose. A small amount of mannose (peak 5) was also detected. Peak 4 (R_L 0.79, solvent a; 0.95, solvent b) yielded labelled fucose on hydrolysis with T.foetus Prep. 1, or on hydrolysis with 2 N HCl for 20 minutes at 100°, and gave radioactive α-Fuc-(1→2)-Gal on alkaline degradation. It was therefore considered to be the H-active trisaccharide α-Fuc-(1→2)-β-Gal-(1→3)-GNAc.[8] Peak 3 (R_L 0.61, solvent a; 0.72, solvent b) yielded only labelled fucose on alkaline degradation and was not hydrolysed by T.foetus Prep. 1. On treatment with T.foetus Prep. 2, labelled fucose was liberated. The behaviour of this compound therefore suggests that it is the Le^a-active trisaccharide β-Gal-(1→3)-[α-Fuc-(1→4)-]-GNAc.[9] Peak 2 (R_L 0.28, solvent a; 0.40, solvent b) co-chromatographed with an authentic sample of the Le^b-active tetrasaccharide, α-Fuc-(1→2)-β-Gal-(1→3)-[α-Fuc-(1→4)-]-GNAc.[10]

The neutral sugars isolated from the incubation mixtures when N-acetyllactosamine was used as the acceptor with the enzyme

preparations from glands No. 20 or 40 or stomach No. 42 gave only one radioactive peak in the trisaccharide area of the chromatogram. (R_L 0.68, solvent a; 0.78, solvent b). Alkaline degradation and enzymic hydrolysis revealed that this peak was a mixture of two components. The formation of labelled α-Fuc-(1→2) Gal on alkaline degradation indicated that one trisaccharide was α-Fuc-(1→2)-β-Gal-(1→4)-GNAc.[8]. The detection of labelled fucose on alkaline degradation, and on treatment with T.foetus Prep. 2, led to the tentative identification of the second trisaccharide as β-Gal-(1→4)-[α-Fuc-(1→3)-]-GNAc. The amounts of radioactivity incorporated into the 2- and 3-linked compounds were calculated from the relative amounts of labelled α-Fuc-(1→2)-Gal (plus any α-Fuc-(1→2)-Tal) and fucose in the alkaline degradation products and from the percentage of the total radioactivity liberated by T.foetus Prep. 1. A small amount of a compound with the R_L value of a difucosyl tetrasaccharide (0.28, solvent a; 0.45, solvent b) was detected in the neutral oligosaccharides formed with N-acetyllactosamine and the enzyme preparation from gland No. 147 (Table 1).

The chromatograms of the neutral sugars obtained from the incubation mixtures when lactose was used as acceptor revealed either one or two radioactive peaks in addition to the labelled origin material and free fucose. With enzyme preparations derived from secretor persons the trisaccharide area (R_L, 0.59, solvent a; 0.72 solvent b) was a mixture of 2'fucosyllactose and a second compound tentatively identified as β-Gal-(1→4)-[α-Fuc-(1→3)-]-Glc.[11]. A slower running substance (R_L 0.32, solvent a; 0.42, solvent b) that co-chromatographed with an authentic specimen of lacto-difucotetraose[5]. was sometimes detected in the reaction products. This same difucosyl compound, in which the second fucosyl residue

is α-linked to the C3 position of glucose was also readily formed when 2'fucosyllactose was used as an acceptor, thus confirming the presence in the enzyme preparations of a 3-fucosyltransferase. Neither 2'fucosyllactose, nor lacto-difucotetraose were detected in the products formed by the enzyme preparations from non-secretors.

Qualitatively the results obtained with submaxillary gland and stomach preparations were the same although the degree of incorporation of $[^{14}C]$ L-fucose varied considerably with different enzyme preparations from either source. However, the 2-fucosyl linkage was always detected in the products formed with enzyme preparations from secretors whereas this linkage was not found in the products formed by the submaxillary gland preparations from non-secretors. These results therefore support the concept[2] that the H gene is responsible for the formation of a 2-α-L-fucosyl-transferase and that the secretor gene Se controls the expression of the H gene.

Table 2. α-L-Fucosyltransferases in Human Stomach Mucosal Tissue

Reaction mixture as in Table 1.

Stomach No	Blood Group	Secretor Status	Acceptor	Probable Identity of Product	Radioactivity c.p.m.	Radioactivity % Total Recovered Counts
42 *	B	Sec	LNB1	2'-FucLNB1	1,100	1
				4-FucLNB1	1,700	2
			LNAc	2'-FucLNAc	3,400	4
				3'-FucLNAc	900	1
			Lact	2'-FucLact	2,200	2
				3-FucLact	3,800	4
45 ⁂	A	Sec	LNB1	2'-FucLNB1	26,800	12
				4-FucLNB1	29,000	13
				2'4-FucLNB1	2,200	1
			Lact	2'-FucLact	5,800	2
				3-FucLact	21,800	9
				2'3-FucLact	2,400	1

⁂ Particle suspension prepared by Method 1
* Particle suspension prepared by Method 2
Abbreviations as in Table 1.

The radioactive trisaccharide in which the fucosyl residue is believed to be attached to the C4 position of the N-acetyl-D-glucosamine was formed with enzyme preparations from tissues of both secretors and non-secretors when lacto-N-biose 1 was added as the sugar acceptor. All the tissues so far examined came from donors who had the Le gene, that is, either Le^a, or Le^a and Le^b, activities were detectable in their secretions. The results are therefore in agreement with the proposal that the Le gene controls the addition of L-fucose to the C4 position of the subterminal N-acetylglucosamine residue in a lacto-N-biose 1 structure to give the Le^a specific grouping. In secretors, where the H gene functions in addition to the Le gene, two fucosyl residues are added to the lacto-N-biose structure to give the tetrasaccharide that has Le^b activity.[10].

The compounds tentatively identified as β-Gal-(1→4)-[α-Fuc-(1→3)-]Glc and β-Gal-(1→4)-[α-Fuc-(1→3)-]GNAc were found in the products given by gland or stomach preparations from both secretors and non-secretors when lactose or N-acetyllactosamine respectively, were used as acceptors. The presence in blood group active glycoproteins of L-fucose linked 1→3 to N-acetylglucosamine was recently reported.[12,13].

Shen, Grollman and Ginsburg[14]. obtained evidence of soluble fucosyltransferases in human milk that correlated with those expected from the secretor status of the donor. The results presented in this paper indicate that in the tissues in which secreted blood group glycoproteins are synthesised, particle-bound α-L-fucosyltransferases are present with the specificities required for the formation of H, Le^a and Le^b specific structures.

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References

1. Watkins, W.M. and Morgan, W.T.J. (1959) Vox Sang., 4, 97.
2. Watkins, W.M. (1966) In Glycoproteins, (Ed. Gottschalk, A.) Elsevier, Amsterdam. p.462.
3. Ginsburg, V. (1966) Methods in Enzymology, 8, 292.
4. Ziderman, D., Gompertz, S., Smith, Z.G. and Watkins, W.M. (1967) Biochem. biophys. res. commun. 29, 56.
5. Kuhn, R. (1957) Angew. Chem. 60, 23.
6. Kuhn, R., Baer, H.H. and Gauhe, A. (1958) Liebigs Ann. Chem., 611, 242.
7. Kuhn, R. and Gauhe, A. (1962) Chem. Ber. 95, 518.
8. Rege, V.P., Painter, T.J., Watkins, W.M. and Morgan, W.T.J. (1964a) Nature, London. 203, 360.
9. Rege, V.P., Painter, T.J., Watkins, W.M. and Morgan, W.T.J. (1964b) Nature, London. 204, 740.
10. Marr, A.M.S., Donald, A.S.R., Watkins, W.M. and Morgan W.T.J. (1967) Nature, London. 215, 1345.
11. Montreuil, J. (1960) J. Bull Soc. Chim. Biol. 42, 1399.
12. Lloyd, K.O., Beychok, S. and Kabat, E.A. (1967), Biochemistry, 6, 1448.
13. Marr, A.M.S., Donald, A.S.R. and Morgan, W.T.J. (1968) Biochem. J. 110, 789.
14. Shen, L., Grollman, E.F. and Ginsburg, V. (1968) Proc. Nat. Acad. Sci. (U.S.A.). 59, 224.