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CHARACTERISATION OF A BLOOD-GROUP A-ACTIVE TETRASACCHARIDE SYNTHESISED BY A BLOOD-GROUP *B* GENE-SPECIFIED GLYCOSYLTRANSFERASE*

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ABSTRACT

The *B* gene-specified α-D-(1→3)-galactosyltransferase, isolated from the serum of a blood-group B individual, was used to catalyse the transfer of *N*-acetyl-D-galactosamine from UDP-*N*-acetyl-D-galactosamine to the blood-group H-active trisaccharide 2′-fucosyllactose. The biosynthetic product had blood-group A activity and its structure was confirmed as α-D-Gal*p*NAc-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-D-Glc by methylation analysis and high-resolution ^{1}H-n.m.r. spectroscopy. This tetrasaccharide was structurally and serologically identical with that made from the same donor and acceptor substrates when the blood-group A gene-specified α-D-(1→3)-*N*-acetylgalactosaminyltransferase was used as the enzyme source. The enzyme encoded by the *B* gene at the blood group *ABO* locus thus has overlapping donor substrate specificity with the enzyme encoded by the allelic *A* gene, and this property confers upon the *B* gene-specified α-D-1→3)-galactosyltransferase the potential to synthesise blood-group A-active structures.

INTRODUCTION

The primary protein product of the human blood-group *A* gene at the *ABO* locus is an α-D-(1→3)-*N*-acetylgalactosaminyltransferase that catalyses the transfer of *N*-acetyl-D-galactosamine from UDP-*N*-acetyl-D-galactosamine to an H-active precursor structure α-L-Fuc*p*-(1→2)-β-D-Gal*p*→R to form an A determinant α-D-Gal*p*Nac-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*→R[1,2]. Under normal physiological conditions the allelic *B* gene-specified glycosyltransferase catalyses the transfer of the D-galactose unit from UDP-D-galactose in α-anomeric configuration to the same orientation in the H structure to form the blood-group B determinant, α-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*→R[3,4]. Recently the serum of blood-group

*Dedicated to Professor Raymond U. Lemieux.

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B individuals was shown, under certain *in vitro* conditions, to exhibit a weak enzyme activity which mimicked the A gene-specified transferase in its utilisation of UDP-*N*-acetyl-D-galactosamine as a donor substrate, and molecules with H determinants as acceptor substrates[5,6]. Sera from more than forty group B donors were found to exhibit this transferase activity, whereas it was not demonstrable in a similar number of sera from group O donors tested under the same conditions. In all the group B sera, the ratio of α-D-galactosyl- to *N*-acetyl-α-D-galactosaminyltransferase activity was approximately the same, and this ratio was maintained in a preparation purified 100 000-fold[5]. These observations, together with substrate-competition experiments and kinetic data on the *B* gene-specified transferase[7], strongly support the inference that the activity in group B serum, measured with UDP-*N*-acetyl-D-galactosamine as donor substrate, is a function of the enzyme encoded by the blood-group *B* gene. In order to confirm that this enzyme transfers the *N*-acetyl-D-galactosamine unit in the same positional linkage and anomeric configuration as the *A* gene-specified transferase, a tetrasaccharide was prepared on a product scale with a purified α-D-(1→3)-galactosyltransferase from group B serum as the enzyme source, UDP-*N*-acetyl-D-galactosamine as the donor substrate, and the low-molecular-weight H-active trisaccharide 2′-fucosyllactose [α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-D-Glc] as acceptor substrate. The characterisation of the biosynthetic tetrasaccharide product by methylation analysis, high resolution ^{1}H-n.m.r. spectroscopy, and serological examination is described herein.

EXPERIMENTAL

Materials and methods. — UDP-D-[U-^{14}C]galactose (307 Ci/mol) and UDP-*N*-acetyl-D-[1-^{14}C]galactosamine (61 Ci/mol) were obtained from Amersham International, Gt. Britain. Unlabelled UDP-*N*-acetyl-D-galactosamine was prepared according to the method of Carlson *et al.*[8]. 2′-Fucosyllactose was purified from human milk as described[9]. The disaccharide α-D-Gal*p*NAc-(1→3)-D-Gal was isolated from the acid hydrolysis products of a blood-group A-active glycoprotein[10]. Methyl 2-acetamido-2-deoxy-α-D-galactopyranoside was prepared by the method of Zilliken *et al.*[11]. The B-active tetrasaccharide α-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-D-Glc was synthesised as described[12]. Blood-group A-active glycoprotein was isolated from an ovarian cyst fluid of a group A secretor patient[13].

Ion-exchange resins AG 1-X4 (200–400 mesh) and AG 50 W-X8 (200–400 mesh) were obtained from Bio-Rad Laboratories Ltd., Watford, Gt. Britain, and Zerolit 225 × 4 (−400 mesh) was purchased from Diamond Shamrock Ltd., London.

Red-cell membranes were prepared by the method of Dodge *et al.*[14] from outdated human group O red cells supplied by the North London Blood Transfusion Centre. The membranes were washed ten times with ~10 vol. of distilled

water and freeze-dried. When required for use, the dried membranes were suspended in water and washed a further three times.

Purification of B *gene-specified* α-D-*(1→3)-galactosyltransferase.* — The *B* gene-specified transferase was purified by adsorption onto group O red-cell membranes, followed by elution of the affinity-bound enzyme with 2′-fucosyllactose[15]. Fresh group B serum (450 mL) was filtered through gauze, centrifuged at 16 000 r.p.m. for 20 min, and M $MnCl_2$ (9 mL), 2M sodium cacodylate buffer, pH 7.5 (45 mL), 10mM UDP-D-galactose (450 μL), and a 0.75% aqueous suspension of group O red cell membranes (45 mL) were added to the supernatant serum. The mixture was kept for 10 min at 4°, and then centrifuged at 16 000 r.p.m. for 10 min. The supernatant was removed, and the sedimented membranes were washed twice with 0.15M NaCl containing 8μM UDP and 16mM $MnCl_2$. The membranes obtained after the second wash were suspended in 5mM 2′-fucosyllactose in 0.15M NaCl (3 mL). The mixture was kept for 1 h at 4°, and then centrifuged at 16 000 r.p.m. for 10 min. The supernatant was removed, and the membranes were eluted a second time with 5mM 2′-fucosyllactose in 0.15M NaCl (3 mL). The supernatants from the two elutions were combined to give a total volume of 6 mL containing the enzyme purified 10 000-fold.

Purification of A *gene-specified* α-D-*(1→3)*-N-*acetylgalactosaminyltransferase.* — The *A* gene-specified transferase was purified from fresh group A_1 serum (250 mL) by the Sepharose-binding procedure of Whitehead *et al.*[16], as modified by Nagai *et al.*[17], to give a final preparation purified 80 000-fold.

Synthesis of α-D-*GalpNAc-(1→3)*-[α-L-*Fucp-(1→2)*]-β-D-*Galp-(1→4)*-D-*Glc with purified* B *gene-specified transferase.* — Tris·HCl buffer, pH 8.0 (300 μmol), $MnCl_2$ (200 μmol), ATP (50 μmol), NaN_3 (75 μmol), 2′-fucosyllactose (150 μmol), unlabelled UDP-GalNAc (30 μmol), UDP-[^{14}C]GalNAc (28 nmol; 2.48 × 10^6 c.p.m.), and bovine serum albumin (100 mg) were added to purified *B* gene-specified α-D-(1→3)-galactosyltransferase (5 mL) to give a total volume of 10 mL. The mixture was incubated for 64 h at 37°. At the end of the incubation period, the mixture was centrifuged at 16 000 r.p.m. for 10 min to remove insoluble material and the supernatant passed through columns (3 ×0.7 cm) of AG 1-X4 (AcO^-) and AG 50W-X8 (H^+) resins. The final eluate and washings were combined, reduced to a small volume by rotary evaporation, and applied to Whatman DE-81 chromatography paper and run in 5:1:1:3 (v/v, solvent *a*) propan-1-ol–pyridine–ethyl acetate–water for 40 h. Marker strips were scanned for radioactivity, and the tetrasaccharide area was cut out and eluted with water. The product was further purified by chromatography on Whatman No. 40 paper in 10:4:3 (v/v, solvent *b*) and 2:1:1 (v/v, solvent *c*) ethyl acetate–pyridine–water, and finally by passage through a column (180 × 1 cm) of Zerolit 225 X-4 (K^+) resin. The yield of tetrasaccharide, based on recovered radioactivity, was 210 μg.

Synthesis of α-D-*GalpNAc-(1→3)*-[α-L-*Fucp-(1→2)*]-β-D-*Galp-(1→4)*-D-*Glc with purified* A *gene-specified transferase.* — Sodium cacodylate buffer, pH 6.0 (200 μmol), $MnCl_2$ (80 μmol), ATP (20 μmol), NaN_3 (30 μmol), 2′-fucosyllactose (150

μmol); unlabelled UDP-GalNAc (30 μmol), UDP-[^{14}C]GalNAc (14 nmol; 1.24 × 10^6 c.p.m.), and bovine serum albumin (40 mg) were added to purified *A* gene-specified α-D-(1→3)-*N*-acetylgalactosaminyltransferase (1.75 mL) derived from group A_1 serum (250 mL). The mixture, in a total volume of 4 mL, was incubated for 64 h at 37°. At the end of the incubation period, the tetrasaccharide was separated from the other components of the reaction mixture and purified as described earlier for the product obtained with the *B* transferase. The yield, based on recovered radioactivity, was 4.64 mg.

Anomeric configuration. — The anomeric configuration of the transferred 2-acetamido-2-deoxy-α-D-galactopyranosyl group was ascertained by treatment with an *N*-acetyl-α-D-galactosaminidase preparation from *Trichomonas foetus*[18]. The tetrasaccharide (75 nmol; 6000 c.p.m.) was incubated at pH 7.0 for 16 h at 37° with 5 mU of enzyme (1 unit hydrolyses 1.0 μmol of *p*-nitrophenyl 2-acetamido-2-deoxy-α-D-galactopyranoside/min at pH 7.0 and 37°). The reaction products were separated by chromatography on Whatman No. 40 paper developed with solvent *b*.

Methylation analysis. — The tetrasaccharide products were each reduced with $NaBH_4$ before methylation analysis. The products were dissolved in 2% $NaBH_4$ and, after 3 h at room temperature, the excess borohydride was eliminated by the addition of acetic acid (~10 μL). The reduced products were repeatedly evaporated with methanol and then passed through columns (0.7 × 3 cm) of AG 50W-X8 resin (H^+).

Methylation was performed by a micromodification of the method of Kuhn *et al.*[19]. The methylated tetrasaccharides were hydrolysed for 16 h at 80° with 0.15M H_2SO_4 in 90% (v/v) acetic acid[20], and separated into neutral and basic fractions by passage through ion-exchange resins[21]. The basic amino sugar methyl ether fraction was examined[21] on an amino acid analyser before and after reduction with $NaBH_4$. The neutral sugar methyl ethers were converted into alditol acetates[20] with $NaBD_4$ as the reducing agent and examined by g.l.c. on (*a*) a 38-m SE 30 capillary column and (*b*) a 39-m SP 2250 capillary column, both temperature-programmed from 160 to 220° at 1°/min. Methylated sugars were identified by their retention times in comparison with standard compounds.

500-MHz ^{1}H-n.m.r. spectroscopy. — The 500-MHz ^{1}H-n.m.r. spectra were recorded with a Bruker AM-500 spectrometer operating in the Fourier-transform mode and equipped with a Bruker Aspect 2000 computer; spectra were accumulated by use of 32k data points and a spectral width of 6 kHz. The probe temperature was 295 K. Chemical shifts (δ) are expressed downfield from sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) and were measured[22] by reference to internal acetone (δ 2.225).

Before ^{1}H-n.m.r. analysis, the oligosaccharides were reduced with $NaBH_4$, and D_2O was repeatedly added and evaporated at room temperature to remove exchangeable protons. The oligosaccharides were finally dissolved in D_2O (400 μL, 99.96 atom % D, Aldrich Chemical Co., Gt. Britain) for examination in 5-mm n.m.r. tubes (Wilmad Glass Co., N.J., U.S.A.).

Serological analysis. — The serological activity of the synthesised tetrasaccharide was measured by inhibition of haemagglutination as previously described[23]. Two anti-A reagents were used; one was a human anti-A obtained from the Blood Group Reference Laboratory, Oxford, Gt. Britain, and the second was a mouse hybridoma-derived monoclonal anti-A 6D4 (ref. 24) kindly supplied by Dr. D. Voak, Regional Transfusion Centre, Cambridge, Gt. Britain. Fresh, washed, blood-group A_2 red cells, in 2% suspension in isotonic phosphate-buffered saline, were used as the indicator cells.

RESULTS

The transfer of *N*-acetyl-D-galactosamine from UDP-*N*-acetyl-D-galactosamine to 2'-fucosyllactose catalysed by the purified blood-group *B* gene-specified α-D-(1→3)-galactosyltransferase yielded a product which was chromatographically indistinguishable from the tetrasaccharide product formed from the same donor and acceptor substrates when the blood-group *A* gene-specified α-D-(1→3)-*N*-acetylgalactosaminyltransferase was used as the enzyme source (Fig. 1). On Whatman No. 40 paper, the tetrasaccharide had $R_{Lactose}$ values of 0.80, 0.45, and 0.63 when developed with solvents *a*, *b*, and *c*, respectively.

Anomeric configuration of transferred 2-acetamido-2-deoxy-D-galactopyranosyl group. — The *N*-acetyl-α-D-galactosaminidase from *T. foetus* com-

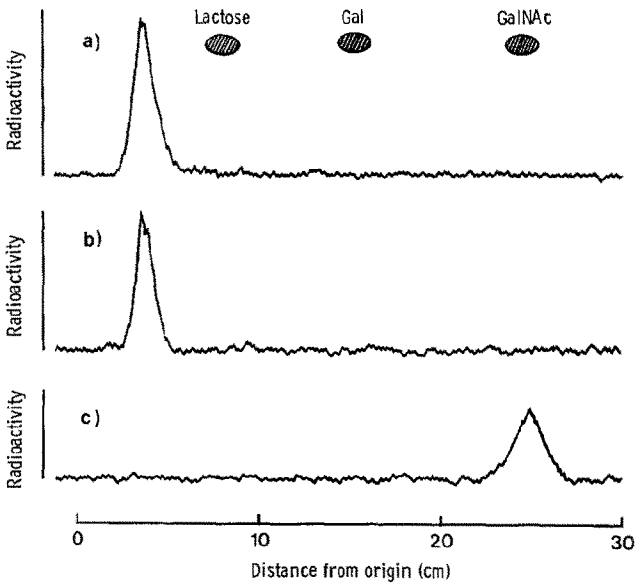

Fig. 1. Paper chromatograms developed in solvent *b* and scanned for radioactivity: a) tetrasaccharide synthesised with *A* gene-specified transferase, b) tetrasaccharide synthesised with *B* gene-specified transferase, and c) product released by treatment, with *N*-acetyl-α-D-galactosaminidase, of tetrasaccharide synthesised with *B* gene-specified transferase.

pletely cleaved the labelled sugar from the tetrasaccharide prepared with the *B* gene-specified transferase, and the released radioactive product cochromatographed with *N*-acetyl-D-galactosamine (Fig. 1). The tetrasaccharide product synthesised with the *A* gene-specified transferase gave the same result.

Methylation analysis. — The tetrasaccharides were reduced with sodium borohydride, permethylated, and hydrolysed, and the hydrolysis products were separated into neutral and basic methyl ethers. The methylated products from the tetrasaccharides synthesised by either the *A* or *B* gene-specified enzymes were identical.

The unreduced 2-amino-2-deoxyhexose methyl ether fractions from the two tetrasaccharide preparations each gave a single peak, on the amino acid analyser, with retention times corresponding to that of standard 2-amino-2-deoxy-3,4,6-tri-*O*-methyl-D-galactose prepared from methyl 2-acetamido-2-deoxy-α-D-galactopyranoside. The reduced 2-amino-2-deoxyhexose methyl ether fractions also gave single peaks on the analyser with the same retention times as the reduced form of the methylated derivative of the methyl glycoside. The methylation results thus confirmed that the sugar unit transferred by both the *A* and *B* gene-specified transferases is unchanged *N*-acetyl-D-galactosamine and that the sugar unit is in a terminal, nonreducing position.

The neutral methyl ether alditol acetates were examined by g.l.c. on SE 30 and SP 2250 columns. The products from each tetrasaccharide gave peaks on both columns with retention times corresponding to standard 4,6-di-*O*-methylgalactose

TABLE I

RELATIVE RETENTION TIMES ON G.L.C. OF METHYLATED GALACTOSE PRODUCTS OBTAINED FROM THE BIOSYNTHETIC TETRASACCHARIDES MADE WITH THE *A* AND *B* GENE-SPECIFIED GLYCOSYLTRANSFERASES

Compounds	*Relative retention time*[a]	
	SE-30 column	*SP-2250 column*
Methyl O-methylgalactopyranoside standards		
2,3,4,6-Tetra-	1.00	1.00
2,3,6-Tri-	1.21	1.29
3,4,6-Tri-	1.25	1.34
2,4,6-Tri-	1.25	1.36
2,3,4-Tri-	1.40	1.46
2,6-Di-	1.43	1.58
4,6-Di-	1.55	1.64
2,3-Di-	1.60	[b]
Biosynthetic tetrasaccharides		
Made with *A*-transferase	1.55	1.64
Made with *B*-transferase	1.55	1.64
2'-Fucosyllactose	1.25	1.34

[a]Relative to methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside. [b]Not determined.

(Table I), and addition of this standard to the methylated products increased the heights of the relevant peaks. In addition the expected peaks corresponding to standard 2,3,4-tri-*O*-methylfucose and 1,2,3,5,6-penta-*O*-methylglucitol were observed. The identity of these neutral methyl ethers was confirmed by g.l.c.–mass spectrometry kindly carried out by Dr. A. Lawson, Clinical Research Centre, Harrow, Gt. Britain. The presence of the 4,6-di-*O*-methylgalactose derivative as the only methylated galactose product demonstrated that, in each tetrasaccharide, the galactose residue was substituted at both O-2 and O-3. Since the acceptor substrate, 2′-fucosyllactose, already has an L-fucose substituent at O-2 of the galactose residue, the transferred *N*-acetyl-D-galactosamine unit is linked to O-3 of this sugar.

^{1}H-N.m.r. spectroscopy. — The 500-MHz ^{1}H-n.m.r. spectra given by the reduced tetrasaccharide products synthesised with the *A* and *B* gene-specified transferases are shown in Fig. 2. The spectra demonstrate that the tetrasaccharides are virtually free from contaminants and that the ^{1}H-n.m.r. spectrum of the compound synthesised with the *B* gene-specified α-D-(1→3)-galactosyltransferase is superimposable upon the spectrum of the tetrasaccharide synthesised with the *A* gene-specified α-D-(1→3)-*N*-acetylgalactosaminyltransferase.

In both spectra, several signals are clearly separated from the bulk resonance of the skeleton protons. These signals were assigned by spin-decoupling experi-

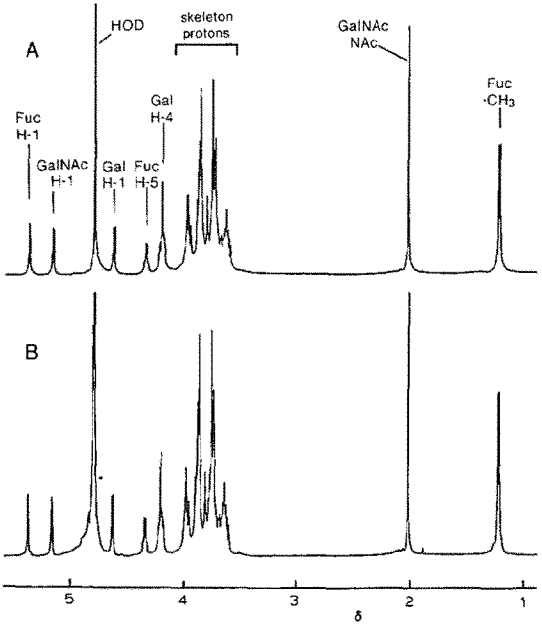

Fig. 2. ^{1}H-N.m.r. spectra of the tetrasaccharide products prepared from UDP-*N*-acetyl-D-galactosamine and 2′-fucosyllactose with: (A) the *A* gene-specified transferase and (B) the *B* gene-specified transferase.

TABLE II

HAEMAGGLUTINATION INHIBITION TESTS WITH BIOSYNTHETIC TETRASACCHARIDES

Compounds	*Minimum concentration of substances giving complete inhibition (μg/10 μl)*	
	Human anti-A	*Mouse monoclonal anti-A*
Synthesised with "A" *transferase*		
α-D-Gal*p*NAc-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-D-Glc	1.5	3
α-D-Gal*p*NAc-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-D-Glcitol	1.5	3
Synthesised with "B" *transferase*		
α-D-Gal*p*NAc-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-D-Glcitol	1.5	3
α-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-D-Glcitol	>100	>100
Controls		
α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-D-Glc	>200	>200
α-D-Gal*p*NAc-(1→3)-D-Gal	1.5	>100
α-D-Gal*p*NAc-(1→3)-D-Galitol	100	>100
D-GalNAc	100	>200
D-GlcNAc	>200	>200
D-Gal	>200	>200
A-Active glycoprotein	0.001	0.002

ments and by comparison with the spectra of the following reference compounds; methyl 2-acetamido-2-deoxy-α-D-galactopyranoside, α-D-Gal*p*NAc-(1→3)-Galitol, and 2′-fucosyllactitol. In the anomeric region, the doublet at δ 5.18 (*J* 3.8 Hz) was assigned to the α anomeric proton of *N*-acetyl-D-galactosamine and the doublet at δ 5.39 (*J* 2.3 Hz) to the α-anomeric proton of L-fucose. These assignments are in agreement with those given to the α-anomeric protons of these two sugars in related compounds by Lemieux *et al.*[25] and Kannagi *et al.*[26], although they are at variance with those proposed by van Halbeek *et al.*[27] on the basis of the coupling constants of the two doublets. The third doublet in the anomeric region at δ 4.65 (*J* 7.7 Hz) was assigned to the β-anomeric proton of D-galactose. Outside the anomeric region, the distinctive signals of H-5 of L-fucose (δ 4.37), H-4 of D-galactose (δ 4.23), the doublet given by CH_3-5 of L-fucose (δ 1.24), and the signal at δ 2.04 given by CH_3 protons of the acetyl group of *N*-acetyl-D-galactosamine are readily distinguishable. Spin-decoupling experiments were used to locate the H-2 signals (H-2 Fuc, δ 3.78; H-2 GalNAc, δ 4.25; and H-2 Gal, δ 3.91).

The ^{1}H-n.m.r. spectra therefore confirm that the tetrasaccharide synthesised by the *B* gene-specified transferase is identical with that synthesised by the *A* gene-specified transferase, and are in complete accord with the assignment of the structure α-D-Gal*p*NAc-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-D-Glc to these compounds.

Serological analysis. — The tetrasaccharides were tested for blood-group A

serological activity in haemagglutination inhibition tests with a human anti-A serum and a mouse monoclonal anti-A reagent (Table II). The total available quantity of the compound synthesised with the *B* gene-specified transferase was reduced with sodium borohydride before ^{1}H-n.m.r. spectroscopy and was tested for serological activity in this form. Although the inhibitory activity of the disaccharide α-D-Gal*p*NAc-(1→3)-D-Gal with the human anti-A serum was destroyed on reduction of the D-galactose unit (Table II), a comparison of the activity of the reduced and unreduced forms of the tetrasaccharide synthesised with the *A* gene-specified transferase demonstrated that conversion of the D-glucose residue in the tetrasaccharide into the sugar alcohol did not alter the inhibitory capacity of the compound. The tetrasaccharide prepared with the α-D-(1→3)-galactosyltransferase as the enzyme source and UDP-*N*-acetyl-D-galactosamine as the sugar donor had the same activity with both anti-A reagents as the corresponding compound synthesised with the *A* gene-specified α-D-(1→3)-*N*-acetylgalactosaminyltransferase. The B-active tetrasaccharide, α-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-D-Glc, which is the product formed by the *B* transferase with 2′-fucosyllactose when UDP-D-galactose is the sugar donor[11], had no detectable inhibitory activity with either anti-A reagent.

DISCUSSION

In the blood-group A determinants in glycoproteins and glycolipids the branched trisaccharide unit α-Gal*p*NAc-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p* is linked to either an *N*-acetyl-D-glucosamine or *N*-acetyl-D-galactosamine unit[28]. However, because of its availability as a constituent of human milk[29], the H-active trisaccharide, 2′-fucosyllactose, has long been used as a low-molecular-weight acceptor substrate in assays for the *A* gene-specified *N*-acetyl-α-D-galactosaminyltransferase[1,2]. The biosynthetic product was earlier characterised as α-D-Gal*p*NAc-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-D-Glc from analysis of partial acid hydrolysis products, the results of periodate oxidation experiments, and by its susceptibility to hydrolysis by *N*-acetyl-α-D-galactosaminidase[1,30]. Until recently, this tetrasaccharide was not known to exist as such in Nature but it has now been shown to be present in urine of some group A secretor individuals[31] and in faeces from breast-fed group A infants[32]. The naturally occurring A-active tetrasaccharide has been subjected to mass-spectrometric analysis[31], but its ^{1}H-n.m.r. spectrum has not been recorded.

The methylation analyses and the ^{1}H-n.m.r. spectra reported herein for the products synthesised from UDP-*N*-acetyl-D-galactosamine and 2′-fucosyllactose, when either the *A* gene-specified α-D-(1→3)-*N*-acetylgalactosaminyltransferase or the *B* gene-specified α-D-(1→3)-galactosyltransferase are used as the enzyme source, confirm the structure previously assigned to the enzymically synthesised tetrasaccharide. Moreover, the identity of the ^{1}H-n.m.r. spectra obtained for the two tetrasaccharides provides unequivocal proof that, although group B individuals

lack an *A* gene, they nevertheless have the inherent capacity to synthesise blood-group A-active structures.

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