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Structures and Activities of Oligosaccharides Produced by Alkaline Degradation of a Blood Group Substance Lacking A, B, H, Le^a, and Le^b Specificities*

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ABSTRACT: Alkaline degradation of an "inactive" blood group substance OG with NaOD–NaBD$_4$ gave a number of compounds which were characterized and each was shown to be identical with one previously isolated and characterized from Le^a substance and from A, B, and H substances. These include 3-hexenetetrol(s), D-galactitol, 2-acetamido-2-deoxy-D-galactitol, and three oligosaccharides β-D-Gal-(1→4)-β-D-GNAc-(1→6?)-3-hexenetetrol(s), β-D-Gal-(1→4)-β-D-GNAc-(1→6)-1,2,4,5,6-hexanepentol(s), and β-D-Gal-(1→4)-β-D-GNAc-(1→6)-[β-D-Gal-(1→3)]-2-acetamido-2-deoxy-D-galactitol. In addition β-D-Gal-(1→3)-2-acetamido-2-deoxy-D-galactitol was isolated and characterized; it corresponds to the small amount of R_L 0.83 previously reported with Le^a substance.

The chromatographic pattern on charcoal from the dialyzable material formed by alkaline borodeuteride degradation of OG was simpler than that previously seen with the A, B, H, and Le^a substances. The amounts of the various compounds isolated from OG were compared with those previously reported from Le^a substance in relation to the mechanism of alkaline degradation and the overall structure proposed for these substances. The data for OG are consistent with the proposed overall structure and indicate that OG can reasonably be considered a precursor of the blood group A, B, H, Le^a, and Le^b substances as well as of the new determinant in which an L-fucosyl residue is linked to C-3 of the GNAc of the type 2 β-D-Gal-(1→4)-β-D-GNAc- chain.

About 1% of individuals have secretions lacking A, B, H, Le^a, and Le^b blood group activities (*cf.* Kabat, 1956; Race and Sanger, 1968). From the secretions of such individuals a glycoprotein similar to the A, B, H, Le^a, and Le^b substances but devoid of these activities has been reported (Watkins and Morgan, 1959; Vicari and Kabat, 1969). According to the genetic scheme advanced by Ceppellini (1959) and by Watkins and Morgan (1959) this "inactive" glycoprotein or precursor should contain both basic types of antigenic determinants, β-D-Gal-(1→3)-D-GNAc (type 1) and β-D-Gal-(1→4)-D-GNAc (type 2), and all groupings that confer A, B, H, Le^a, or Le^b activity should be missing. These two types of determinants are joined by β-(1→3) and β-(1→6) linkages to a galactose which is in turn linked to several additional sugars; an overall structure for the oligosaccharide moiety has been advanced (Lloyd *et al.*, 1968; Lloyd and Kabat, 1968). The genetic scheme considers only the four gene systems (ABO, Hh, Lele, Sese) responsible for the synthesis of the outer terminal sequences of the carbohydrate chains in the blood group glycoproteins of

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body fluids and secretions. If the biosynthesis of the interior portions of these chains is determined by several additional genes, the existence of several precursors might be hypothesized, each produced by a block at any point in the interior of the carbohydrate chain. This may in part explain the reports by different workers of inactive blood group substances with different characteristics. Thus, Watkins and Morgan (1959) have given analytical data on one such preparation (Fl) which cross-reacted very extensively with type XIV antipneumococcal serum and contained only 1.6% fucose. Carlson (1968) has isolated a sialic acid rich glycoprotein which had no detectable blood group activity and did not react with type XIV antipneumococcal serum. Kobata *et al.* (1969) have reported a blood group inactive glycoprotein which could have resulted as a consequence of the absence of the enzyme responsible for the attachment to the main chain of the galactose to which the type 1 and type 2 determinants are linked, since specific fucosyltransferases responsible for H and for Le^a activity were present in the milk of the individual.

Recently, the preparation and characterization of a glycoprotein from ovarian cyst fluid of a Nigerian (OG) which was devoid of blood group A, B, H, Le^a, and Le^b activity have been described (Vicari and Kabat, 1969). It also had a low fucose content (2.5%), showed strong cross-reactivity with type XIV antipneumococcal horse serum and did not precipitate with goat anti-Le^a serum.

The present study describes the isolation of some oligosaccharides after alkaline borodeuteride degradation of the "inactive" OG substance and elucidation of their structures. These are compared with the structures of oligosaccharides previously isolated from Le^a and from A, B, H substances and with the overall structure proposed for the carbohydrate portion of the water-soluble blood group A, B, H, Le^a, and Le^b substances (Lloyd *et al.*, 1968; Lloyd and Kabat, 1968).

Methods

Analytical Methods. Colorimetric methods for the analysis of nitrogen, hexosamine, *N*-acetylhexosamine, galactosamine, methylpentose (fucose), and hexose (galactose) have been described previously (Kabat, 1961; Lloyd *et al.*, 1966, 1968).

Gas–Liquid Chromatography. Oligosaccharides were hydrolyzed and analyzed for their constituent sugars by gas–liquid partition chromatography of their alditol acetate derivatives (Sawardecker *et al.*, 1965; Björndal *et al.*, 1967; Albersheim *et al.*, 1967; Perry and Webb, 1968). Oligosaccharide (0.1–1.0 mg) was hydrolyzed for 2 hr at 100° in 2 N HCl or in 2 N trifluoroacetic acid and erythritol and/or xylose were added as internal standards. Trifluoroacetic acid was then removed by evaporation to dryness *in vacuo* while HCl was neutralized with a slight excess of silver carbonate and the suspension centrifuged or filtered. Reduction was performed with sodium borohydride (10 mg) at room temperature for at least 3 hr. After neutralization of excess borohydride with acetic acid and concentration *in vacuo*, boric acid was removed by codistillation with methanol and the product acetylated with acetic anhydride–pyridine 1:1 at 100° for 20 min. The acetylation mixture was diluted with water, evaporated to dryness, and dissolved in chloroform. Gas–liquid chromatographic separation was carried out on a glass column containing 3% (w/w) of ECNSS-M on Gas Chrom Q (100–200 mesh). The temperature of the column was held at 190° for 25–30 min with the neutral sugars and then raised to 215° for the hexosamines.

Paper Chromatography. The following solvent systems were used: 1-butanol–pyridine–water (6:1:1), solvent 1; 1-butanol–pyridine–water (6:4:3), solvent 2; with galactose and lactose (L) as reference compounds.

Column Chromatography. Methods for charcoal column chromatography and gel filtration on Bio-Gel P 2 have been described (Lloyd *et al.*, 1966).

Periodate Oxidation. Quantitative oxidation by periodate for structural analysis of oligosaccharides was carried out as described previously (Schiffman *et al.*, 1962; Lloyd *et al.*, 1966).

Methylation. Oligosaccharides (2 mg) were methylated with methyl iodide and Ag_2O in dimethylformamide followed by methyl iodide and BaO in dimethylformamide as previously described (Kuhn *et al.*, 1955, 1958; Lloyd *et al.*, 1966). Methyl glycosides of the methylated sugars were obtained by treating with 2.5% HCl in methanol for 18 hr at 60°. The neutral methyl sugars were analyzed as their methyl glycosides by gas–liquid chromatography on (1) ECNSS-M (3%) on Gas Chrom Q at 145° and (2) neopentyl glycol succinate (10%) on acid-washed Chromosorb W at 150°.

Immunochemical Methods. Inhibition of precipitation was studied using 40 μl of type XIV horse antipneumococcal serum (H 635, 139 bleeding) (Kabat, 1962) with 10.4 μg of one of the purified fractions (OG.1) of the inactive blood group substance.

Experimental Section and Results

Degradation of Blood Group Inactive Substance and Purification of Oligosaccharides. Treatment of Blood Group Inactive Substance with NaOD–NaBD$_4$. The blood group substance was isolated from the ovarian cyst fluid of a Nigerian patient (OG). Four purified fractions (OG.1, OG.2, OG.3, and OG.4) had been obtained by pepsin digestion followed by extraction in 90% phenol and precipitation from phenol by different concentrations of ethanol as previously described (Vicari and Kabat, 1969). OG.1 (85.3 mg), OG.2 (86.9 mg), OG.3 (392 mg), and OG.4 (295 mg) were combined (total 860 mg) and treated with 0.2 M NaOD and 1% $NaBD_4$ in D_2O at room temperature for 7 days as previously described (Kabat *et al.*, 1965; *cf.* Schiffman *et al.*, 1964). After exhaustive dialysis 275 mg (32%) of the product was nondialyzable while 547 mg (64%) was isolated in the dialysate. Moreover, 27.8 mg (3%) of galactitol was isolated and identified from the first dialysate during the process of desalting through retardion.[1] In Table I, the analytical data on the dialyzable and nondialyzable fractions are given in comparison with one of the OG fractions of the starting material.

Fractionation of Dialyzable Products. The fractionation followed the procedure already used for the corresponding A, B, H, and Le^a oligosaccharides. Fractions were purified until the products met the criteria of purity described previously (Lloyd *et al.*, 1966). The dialyzable material (519 mg) was first fractionated by chromatography on a charcoal

[1] Manufactured by Dow Chemical Co., Midland, Mich.

TABLE I: Analytical Data on Inactive Cyst Substance OG.3 and Products of Its Alkaline Degradation.

Fraction	Per Cent Composition					
	N	Methyl-pentose (Fucose)	Hexose (Galactose)	Hexosamine	Glucosamine[a]	Galactosamine
OG.3	6.8	2.4	32.5	29.0	18.7	10.3
Alkaline degradation						
Dialyzable	3.7	1.9	28.2	19.0	17.2	1.8
Nondialyzable	9.2	1.3	16.3	15.1	10.7	4.4

[a] Hexosamine minus galactosamine.

(160 g)–Celite (80 g) column (Whistler and Durso, 1950) and eluted with an ethanol gradient. The elution curve is shown in Figure 1; the tubes were pooled as shown. Yields of the main fractions and components present are given in Table II.

Fraction 621–655 after evaporation gave crystals. Recrystallization from methanol–water of this compound and of the galactitol (27.8 mg) isolated and purified from the first dialysate during desalting gave identical products (9.3 and 19.9 mg, respectively). The melting points were 188.5–189° and 187–187.5°. Mixture melting points with authentic galactitol showed no depression.

Fractions 746–812, 813–850, and 851–920 were further resolved by preparative paper chromatography in solvent 2. Compounds with similar R values were combined and further purified. The following compounds were isolated: (1) R_{Gal} 1.30 (42.6 mg) was purified by gel filtration through a Bio-Gel P2 column (27.7 mg). Crystallization from methanol–water gave 15.9 mg (mp 174–175°); (2) R_{Gal} 1.61 from fraction 813–850 (4.0 mg) was subjected to paper chromatography in solvent 1 and combined with R_{Gal} 1.61 from fraction 746–812 (10.7 mg). The pooled material was further purified by gel filtration through Bio-Gel P2. Final yield was 7 mg.

Fraction 921–960 on gel filtration through Bio-Gel P2 gave 9.8 mg of R_{Gal} 0.87.

Fractions 1031–1080 and 1081–1130 were shown to contain a number of components by paper chromatography in solvent 2. Preparative paper chromatography in the same solvent gave the following: (1) R_L 0.68 (26.4 mg) which was passed through a Bio-Gel P2 column (13.2 mg) and (2) R_L 0.44 (41.1 mg) which was first chromatographed on a charcoal column (23.3 mg) and finally purified by gel filtration through a Bio-Gel P2 column (21.3 mg).

Fractions 1131–1175 and 1176–1200 also contained a number of components. By further purification on a charcoal column two main fractions having R_L 1.1 were isolated (44.6 mg and 14 mg); they were chromatographed separately on Bio-Gel P2. From the first fraction 34.3 mg was obtained; the single peak from the second was divided in two parts (5.1 mg and 4.2 mg, respectively). All products were the same oligosaccharide.

Determination of Structures of Oligosaccharides. The analytical properties of isolated oligosaccharides are listed in Table III. In Table IV the [α] values are given in comparison with the oligosaccharides with similar R_L values isolated (Lloyd *et al.*, 1968) from Le[a] substance.

Reduced Monosaccharides. The crystalline products from the retardion treatment of the first dialysate and from fractions 621–655 were shown to be galactitol by melting point and mixture melting point and by their chromatographic behavior (R_{Gal} 1.1 in solvent 1 and R_{Gal} 1.06 in solvent 2).

R_{Gal} 1.3 from fractions 746–812, 813–850, and 851–920 was identified as *N*-acetylgalactosaminitol as follows: (a) mp 174–175°, mixture melting point showed no depression; (b) R_{Gal} 1.84 and 1.61 in solvents 1 and 2 identical with standard; (c) N 6.2% (theory 6.3%); (d) $[\alpha]_D^{20}$ −41.6° (authentic sample −40.2); (e) gas–liquid chromatography of acetylated derivative gave identical retention time as standard. Mixture with acetylated *N*-acetylglucosaminitol gave two peaks, but mixture with acetylated *N*-acetylgalactosaminitol gave only one.

R_{Gal} 1.61 from fractions 746–812, 813–850, and 851–920 had a chromatographic mobility in solvent 1 and solvent 2 (R_{Gal} 3.5) identical with the 3-hexenetetrols previously isolated from the products of alkaline degradation of human A, B, H, Le[a], and hog (A + H) substances. The compound decolorized

TABLE II: Fractions from Charcoal Column of Dialyzable Product (519 mg).

Charcoal Column Fraction	Yields (mg)	Components Identified
621–655	29.7	Galactitol
746–812	55.2	*N*-Acetyl-D-galactosaminitol, hexenetetrols
813–850	14.8	*N*-Acetyl-D-galactosaminitol, hexenetetrols
851–920	20.6	*N*-Acetyl-D-galactosaminitol
921–960	19.0	R_{Gal} 0.87[a]
1031–1080	30.5	R_L 0.44, R_L 0.68
1081–1130	84.4	R_L 0.44, R_L 0.68
1131–1175	112.1	R_L 1.1
1176–1200	21.3	R_L 1.1

[a] All R values in solvent 2 (BuOH–pyridine–H_2O, 6:4:3).

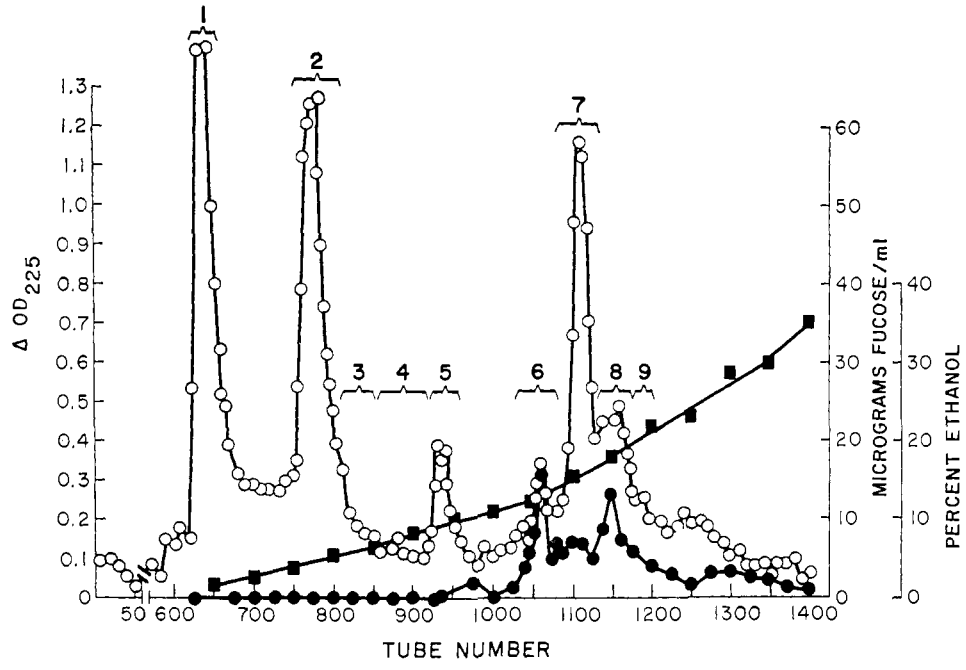

FIGURE 1: Fractionation on charcoal–Celite column of dialysate (519 mg) from treatment of OG with NaOD–$NaBD_4$: (○) periodate consumption ΔOD_{225}; (●) fucose; (■) ethanol concentration.

TABLE III: Analytical Properties of Isolated Oligosaccharides.

Oligosaccharide	Yield (mg)	Unsatn Test	Per Cent Composition						Mole Ratios				HexNAc/HexN
			N	Fuc	Gal	HexN	HexNAc[a]	GalN	N	Gal	HexN	HexNAc	
OG R_{Gal} 0.87	9.8	−	3.0	1.1	34.0	0.6	0.7	0.8	1.14	1.00			
Calcd			3.6	0	46.8	0	0	0	1.0	1.0			
OG R_L 1.1	43.6	+	2.3	1.7	35.2	32.3	37.5	2.3	0.86	1.00	0.92	0.87	0.95
Calcd			2.7	0	35.1	34.8	43.2	0	1.0	1.0	1.0	1.0	1.0
OG R_L 0.68	13.2	−	2.8	3.0	33.1	29.3	31.7	2.2	1.1	1.00	0.89	0.78	0.89
Calcd			2.6	0	34.0	33.8	41.5	0	1.0	1.0	1.0	1.0	1.0
OG R_L 0.44	21.3	−	3.5	1.3	47.9	21.3	26.5	1.6	1.88	2.00	0.88	0.89	1.0
Calcd			3.7	0	48.0	23.9	29.5	0	2.0	2.0	1.0	1.0	1.0

[a] With the colorimetric methods used for HexNAc, GalNAc gives 33% of the color of GNAc, while in that for HexN, both give equal colors. Thus mole ratios of 1.0, 0.33, and 0.67 HexNAc/HexN indicates all GNAc, all GalNAc, and equimolar proportions of both.

bromine and potassium permanganate solution. Identification was also performed by gas–liquid chromatography of the acetylated derivative: retention time relative to erythritol was 3.28 for the unknown and 3.27 for the authentic 3-hexenetetrol.

R_{Gal} 0.87. Analytical data and gas–liquid chromatography of the alditol acetate derivatives after hydrolysis of the compound indicated that this was a disaccharide containing galactose and *N*-acetylgalactosaminitol. The compound consumed 3.0 moles of periodate and released 0.9 mole of formaldehyde and 1.4 moles of formic acid per mole. However the galactose content (Table III) accounts for only 80% of the calculated value and the N was somewhat low indicating that some inert weight might be present. Recalculation of the periodate oxidation data based on galactose gave 3.6 moles of periodate, 1.1 moles of formaldehyde, and 1.7 moles of formic acid per mole of compound. Thus, correcting for the inert weight would suggest a 1→3 linkage.

R_L 1.1. Analytical data, optical rotation, chromatographic properties, and periodate oxidation showed that this oligosaccharide is identical with Lewis R_L 0.96. The presence of 3-hexenetetrol(s) at the reducing end demonstrated by the ability to decolorize bromine and permanganate solutions was further confirmed by gas–liquid chromatography of the hydrogenated and hydrolyzed oligosaccharide. To

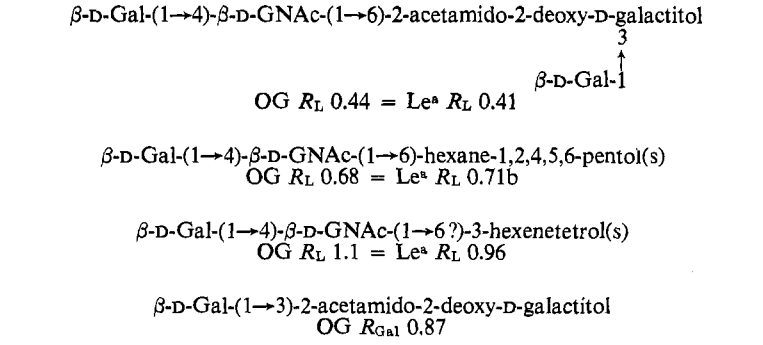

FIGURE 2: Proposed structures of oligosaccharides from OG substance.

prevent destruction of the hexenetetrol(s) during the subsequent hydrolysis, hydrogenation was carried out for 4–5 hr under hydrogen pressure using palladium on charcoal powder as catalyst. The retention time of the 1,2,5,6-hexanetetrol(s) relative to erythritol was 3.2 for both the standard and the unknown. The gas–liquid chromatography also showed the presence of galactose and glucosamine. Paper chromatography in solvent 1 after partial acid hydrolysis, in 0.03 M HCl for 2 hr, showed the presence of a spot with the mobility of β-D-Gal-(1→4)-D-GNAc. After methylation 2,3,4,6-tetra-*O*-methylgalactose was identified as the methyl glycoside; retention time relative to methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside—column (1) 1.3 (standard 1.3), (2) 1.19 (standard 1.19); no other neutral sugars were present.

R_L 0.68. Gas–liquid chromatography of a hydrolysate of this compound demonstrated the presence of both hexenetetrol(s) and hexane-1,2,4,5,6-pentol(s) together with galactose and *N*-acetylglucosamine. Therefore the material was not pure and this explains the disagreement in the optical rotation values between this compound and the Lewis oligosaccharides with similar R_L (Table IV). Methylation analysis of the mixture gave the methyl glycoside of 2,3,4,6-tetra-*O*-methyl-D-galactose; retention time relative to methyl 2,3,4,6-tetra-*O*-methyl-D-glucoside—column (1) 1.2 (standard 1.31), (2) 1.18 (standard 1.18). It is very likely that the main oligosaccharide in the mixture was a trisaccharide identical with Lewis R_L 0.71b. The higher levorotation could be contributed by a small amount of fucose containing oligosaccharide since the material contained 3% of methylpentose perhaps present in a compound similar to the fucose containing Lewis R_L 0.71a (Tables III and IV). A small amount of tri-*O*-methyl-L-fucose was identified as the methyl glycoside by gas chromatography.

R_L 0.44. Analysis of this compound, periodate oxidation data, and optical rotation values are all in favor of the identity of this compound with R_L 0.41 from Lewis substance. Methylation analysis showed the methyl glycoside of 2,3,4,6-tetra-*O*-methylgalactose as the only neutral sugar; retention time relative to methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside—column (1) 1.3 (standard 1.3), (2) 1.18 (standard (1.19)). The tentative structure previously given (Lloyd *et al.*, 1968) is indicated in Figure 2 except that the Gal–GNAc linkage is accepted as (1→4) since Marr *et al.* (1968) have isolated β-D-Gal-(1→4)-β-D-GNAc-(1→6)-D-GalNAc by alkaline degradation of Le^a substance from ovarian cyst fluid; the structures of the other oligosaccharides described above are also given. In addition to the oligosaccharides isolated and characterized, considerable amounts of materials with very low R_L values on paper were obtained. These could not be adequately fractionated to give homogeneous products. This has also been the case with the other blood group substances studied earlier.

Immunochemical Studies. The lack of A, B, H, Le^a, and Le^b blood group activity of the OG substance and its strong cross-reactivity with type XIV antipneumococcal horse serum have been reported previously (Vicari and Kabat, 1969). Figure 3 shows the inhibition by the various oligosaccharides of precipitation of OG.1 with type XIV antipneumococcal serum. OG R_L 1.1 and Le^a R_L 0.96 are equally active and are the best inhibitors in this system, closely followed by OG R_L 0.44 and Le^a R_L 0.41. Le^a R_L 0.71b is almost as good as Le^a R_L 0.41 and OG R_L 0.44, but OG R_L 0.68 which has some impurity is somewhat less active, as would be expected. The results are essentially the same as those reported (Lloyd *et al.*, 1968) and the slight relative differences are probably due to the use of OG rather than Le^a substance in the cross-reaction studies since the former cross-reacts to a greater extent (Vicari and Kabat, 1969). As expected from previous data (Kabat, 1962) β-D-Gal-(1→4)-β-D-GNAc-(1→6?)-3-hexenetetrol(s) (OG R_L 1.1 and Le^a R_L 0.96) and β-D-Gal-(1→4)-β-D-GNAc-(1→6)-1,2,4,5,6-hexanepentols (OGR_L 0.68 and Le^a R_L 0.71b) are better inhibitors than β-D-Gal-

TABLE IV: Specific Optical Rotation of OG and Le^a Oligosaccharides.[a]

Oligosaccharide	Wavelength (nm)				
	589	578	546	436	365
OG R_{Gal} 0.87	−31	−31.6	−36.8	−64.0	−107.0
OG R_L 1.1	−18	−18.7	−21.8	−40.4	−70.2
Le^a R_L 0.96	−19.8	−19.8	−22.4	−38.2	−68.5
OG R_L 0.68	−24.8	−24.1	−29.0	−52.4	−87.6
Le^a R_L 0.71a	−56.4	−56.4	−65.4	−111.0	−175.0
Le^a R_L 0.71b	−7.6	−12.1	−15.2	−30.4	−53.1
OG R_L 0.44	−42.3	−43.0	−49.8	−86.8	−143
Le^a R_L 0.41	−41.3	−42.8	−48.8	−87.0	−142

[a] Le^a compounds were those of Lloyd *et al.* (1968).

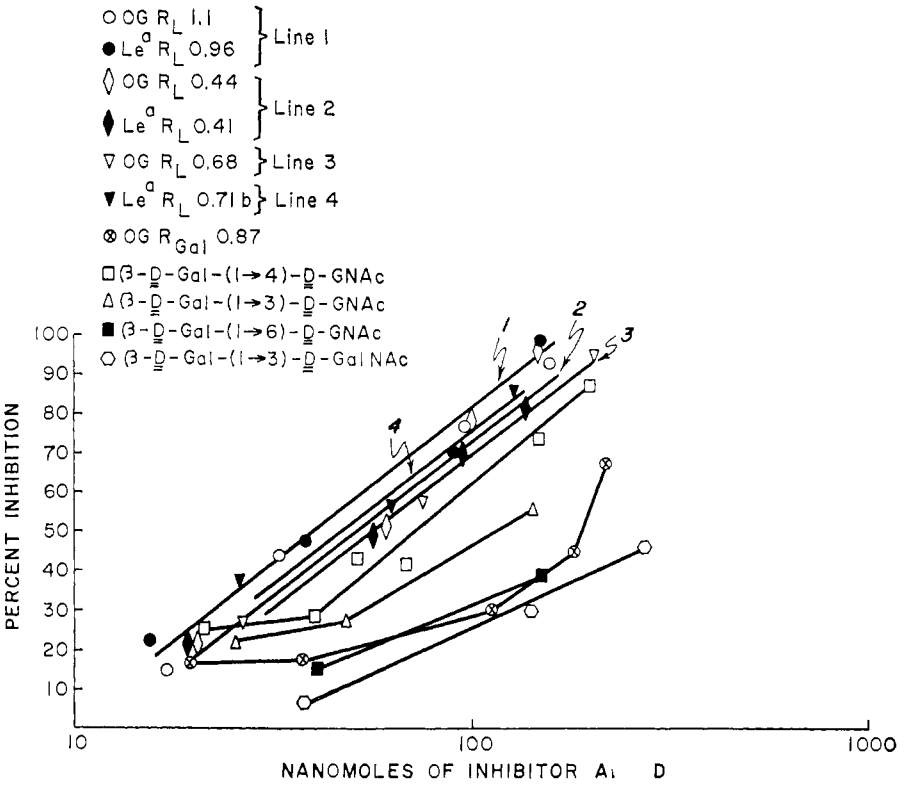

FIGURE 3: Inhibition by oligosaccharides of the cross-reaction between horse antipneumococcal type XIV serum and OG substance-serum H 635 '39 bleeding (40 μl), OG. 1 (10.4 μg); total volume 300 μl.

(1→4)-D-GNAc, demonstrating that the antibody combining site in the type XIV cross-reaction involves at least 2 sugars plus the second β linkage. The superiority as an inhibitor of β-D-Gal-(1→4)-D-GNAc over β-D-Gal-(1→3)-D-GNAc and β-D-Gal-(1→6)-D-GNAc (Watkins and Morgan, 1956; Allen and Kabat, 1959) was confirmed. β-D-Gal-(1→3)-D-*N*-acetylgalactosaminitol (R_{Gal} 0.87) and β-D-Gal-(1→3)-D-GalNAc (Flowers and Shapiro, 1965) were poor inhibitors in this system.

Discussion

Alkaline borodeuteride degradation of the OG substance liberated 65% of dialyzable material, almost twice as much as was obtained from Le^a substance (Lloyd *et al.*, 1968) under similar conditions. Analyses for various constituents in the dialyzable and nondialyzable fractions and calculation of the total recovery gave the following values for per cent recovery: weight, 96; N, 79; methylpentose, 68; Gal, 72; hexosamine, 58; *N*-acetylhexosamine, 77; galactosamine, 21; indicating that selective destruction of GalNAc had occurred as a consequence of alkaline elimination from serine and threonine (Kabat *et al.*, 1965; Anderson *et al.*, 1963). The dialyzable material also gave a much simpler chromatographic pattern on charcoal–Celite chromatography (Figure 1) than had hitherto been encountered with the other blood group substances (Lloyd *et al.*, 1966, 1968). This is not surprising since the other blood group substances had a high fucose content and most of the oligosaccharides isolated were fucose containing, while OG has a very low fucose content. Three of the oligosaccharides were identical in chemical and physical properties, in structure, and in cross-reactivity with type XIV antipneumococcal horse serum with those obtained from the Le^a substance—OG R_L 0.44, R_L 0.68, and R_L 1.1 corresponding to Lewis R_L 0.41, R_L 0.71b, and R_L 0.96. The fourth oligosaccharide, OG R_{Gal} 0.87, probably corresponds to the small amount (5 mg) of Lewis R_{Gal} 0.83 which also had been found to contain galactose and *N*-acetylgalactosaminitol.

Comparison of the yields of oligosaccharide isolated from OG and from the Le^a substance (Lloyd *et al.*, 1968) is of some interest. Although only 860 mg of OG was available for alkaline borodeuteride degradation as compared with 3 g of Le^a substance, OG gave 547 mg of dialyzable material as compared with 1.1 g from Le^a. Thus losses in work-up and isolation might be expected to be greater with OG. Despite this 9.8 mg of OG R_{Gal} 0.87 was isolated as compared to 5 mg of Le^a R_{Gal} 0.83. OG R_L 1.1 amounted to 8.3% while Le^a R_L 0.96 was only 2.8% of the respective dialysates. Similarly the percentages of OG R_L 0.44 and Le^a R_L 0.41 were 4.1 and 1.1, respectively. Only OG R_L 0.68 occurred in lower proportion (2.5%) than its counterpart Le^a R_L 0.71b (4.2%).

From the mechanism of alkaline borohydride degradation proposed (Lloyd *et al.*, 1968), OG R_L 0.44 and Le^a R_L 0.41 represent incomplete chains attached by GalNAc to serine and threonine, and the higher yield obtained with OG would suggest that this precursor type substance had more of these incomplete chains. OG R_L 1.1 and Le^a R_L 0.96 which have a reducing end terminated by 3-hexenetetrols would

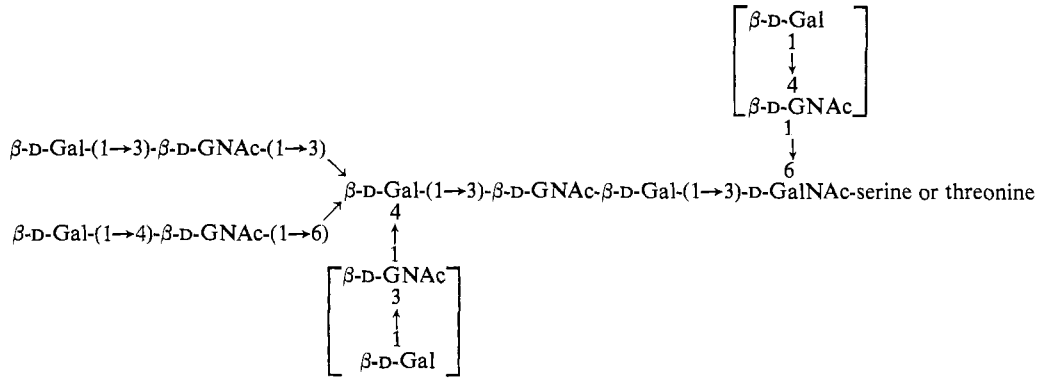

FIGURE 4: Proposed overall composite structure for OG oligosaccharide moiety. The structure presented is based on the earlier composite structure for the A, B, H, Le^a, and Le^b substances (Lloyd *et al.*, 1968; Lloyd and Kabat, 1968), and shows the relationships of the type 1 and type 2 determinants upon which these antigens are built. It is subject to all of the limitations considered earlier. As in the earlier studies, incomplete chains are present and can result from incomplete biosynthesis or by degradation in the cyst cavity. Moreover, the bracketed substitution on carbon 4 of the 3,4,6-linked galactose could be a galactose on some residues; whatever the residue, it must be a sequence capable of giving galactitol on peeling.

have arisen from elimination of the substituents on C-3 and C-4 from the 3,4,6-linked galactosyl residue in the proposed overall structure of the blood group substances while OG R_L 0.68 and Le^a R_L 0.71b with a reducing terminus of 1,2,4,5,6-hexanepentols would have been formed if the galactose were substituted on C-3 but not on C-4. The higher yield of the hexenetetrol-containing compound (8.3%) as compared with the hexanepentol-containing compound from OG (2.5%) suggests that most of the type 2 chains were originally substitued on both C-3 and C-4 rather than only on C-3 and hence carry type 1 and type 2 determinants. This is of especial significance since no β-D-Gal-(1→3)-β-D-GNAc-(1→3)-[β-D-Gal-(1→4)-β-D-GNAc-(1→6)]-D-galactitol (R_L 0.17) was isolated from OG while 17.4 mg was isolated from Le^a substance (Lloyd *et al.*, 1968). Since only about half as much dialyzable material from OG was available one would only expect to isolate about 7 or 8 mg if the degradation had proceeded in the same way and such a small quantity might have been difficult to isolate and purify. This compound would be formed only from chains with the galactose substituted on C-3 and C-6 but not on C-4 which were reduced by the borodeuteride before elimination could occur.

When one compared the proportions of the three other substances isolated from OG and from Le^a—D-galactitol, *N*-acetyl-D-galactosaminitol, and 3-hexenetetrols—one finds the results reported in Table V. It is evident that the amounts of D-galactitol are not very different. However OG yielded more *N*-acetyl-D-galactosaminitol (34% of the total GalNAc) and less 3-hexenetetrols than did the Le^a substance. The higher proportion of the former suggests that more incomplete chains of terminal GalNAc are present in the OG than in the Le^a substance while the lower proportion of free 3-hexenetetrol(s) indicates that OG contains fewer chains in which the galactose is linked only C-3 and C-4 but not on C-6. Thus the lower proportion of 3-hexenetetrol(s) and oligosaccharide with a reducing end terminated by 1,2,4,5,6-hexanepentols and the absence of the branched oligosaccharide R_L 0.17 terminated by galactitol would be expected to be accompanied by a higher proportion of oligosaccharide with a reducing end of 3-hexenetetrols (OG R_L 1.1), as is the case.

TABLE V: Per Cent of Dialyzable Material.

	D-Galactitol	*N*-Acetyl-D-galactos-aminitol	3-Hexene-tetrols
OG	5.6	3.1	1.4
Le^a	7.6	1.3	5.3

From these considerations the overall composite structure of the oligosaccharide moiety of OG would be consistent with that proposed for the Le^a substance (Lloyd *et al.*, 1968) but the fucose side chains would be lacking (Figure 4). As with the other blood group substances there would be small amounts of incomplete chains but a substantial proportion (*ca.* 50%) of terminal nonreducing chains of *N*-acetyl-D-galactosamine (Lloyd and Kabat, 1968).

It is of interest that no fucose containing oligosaccharides were isolated. This is not surprising since the fucose content of OG was so low. From the analytical compositions of the other blood group substances, 1 or 2 moles of fucose may be present (Lloyd and Kabat, 1968) which are not accounted for in the overall structure. The fucose in OG might be of this type.

From the overall composite structure in Figure 4 OG can reasonably be said to be a precursor of the A, B, H, Le^a, and Le^b determinants as well as of the new determinant (Lloyd and Kabat, 1968; Kobata and Ginsburg, 1969) in which an L-fucosyl residue is linked glycosidically to C-3 of the GNAc of the type 2 chain. That OG is a precursor is further supported since Jarkovsky *et al.* (1970) have successfully used the OG substance as an acceptor onto which fucose as L-fucose has been transferred by a fucosyltransferase from milk to give an Le^a active product.

Although the OG substance clearly qualifies as a precursor substance, whether it was synthesized as such by the individual or whether it arose from degradation in the cyst cavity

is not known. Data were unfortunately not obtained on the secretor status of Mrs. OG although she was known to be of blood group B.

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