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PREPARATIVE, AFFINITY CHROMATOGRAPHY OF SHEEP GASTRIC-MUCINS HAVING BLOOD-GROUP Ii ACTIVITY, AND RELEASE OF ANTIGENICALLY ACTIVE OLIGOSACCHARIDES BY ALKALINE-BOROHYDRIDE DEGRADATION

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ABSTRACT

A large number of oligosaccharide fractions having blood-group Ii activity were obtained by degradation with alkaline borohydride of sheep gastric-glycoproteins which had been enriched for these blood-group activities by affinity chromatography on an anti-I adsorbent column The approximate molecular weights of the oligosaccharides were estimated from their elution profiles on Bio-Gel P4 and from monosaccharide compositions The fractions of smallest molecular weight with both I and i activities were mixtures of hexa- to octa-saccharides From a comparison of the inhibitory activities of these with fractions of higher molecular weight and with synthetic oligosaccharides, it was concluded that the antigenic determinant recognised by anti-I Ma is a trisaccharide, whereas those recognised by other types of anti-I and by anti-i antibodies are longer than trisaccharides

INTRODUCTION

Human, monoclonal autoantibodies having I and i specificities recognise antigenic determinants on oligosaccharide precursors of the major blood-group antigens[1,2] and of certain erythrocyte gangliosides[3–6] The monoclonal autoantibodies of individual donors apparently recognise different oligosaccharide domains on branched (anti-I) and linear (anti-i) precursor-chains In order to define precisely the antigenic determinant recognised by each antibody, it is necessary to purify and characterise I- and i-active oligosaccharide chains of various sizes Mucus glycoproteins are ideal sources of oligosaccharides for antigenic analysis, since their oligosaccharide chains can be readily released by degradation with alkaline borohydride[7] In the course of antigenic analyses of glycoproteins from human and animal origins we observed that sheep gastric-glycoproteins are an abundant source of blood-group I and i antigens[8,9] The highest blood-group I activities were found in glycoproteins lacking in blood-group A and H activities, and both the I and i activities could be specifically enriched by affinity chromatography using a column of insolubilised anti-I antibody

In the present studies, a pool of sheep gastric-glycoproteins selected for strong

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blood-group Ii activities were further enriched for Ii antigens by preparative scale, affinity chromatography and subjected to alkaline degradation in the presence of tritiated sodium borohydride Chromatography on Bio-Gel P4 of oligosaccharides thus released yielded 21 fractions. 17 of which showed blood-group i and/or I activities by radio-immunoassays

MATERIALS AND METHODS

Preparation of sheep gastric-glycoproteins having blood-group Ii activity

Fresh. sheep stomachs (abomasa) were obtained in batches of thirty from a local abbatoir In a screening procedure to select I-active samples for extraction, several strips were cut from each mucosa homogenised in isotonic saline (1 ml/g of wet tissue) at full speed for 1 min on an MSE blender, and centrifuged at 2,000*g* for 10 min, and the supernatant solution was tested in haemagglutination-inhibition assays[10] at 4° with anti-I serum Low[5] Glycoprotein extracts were prepared as described previously[9], from those sheep stomachs (~1 in 4 of those screened) which gave haemagglutination inhibition at dilutions of 1 4 or greater Two pools of Ii-active glycoproteins designated pool A (32 individual extracts) and pool B (8 individual extracts) of 3 3 and 0 9 g, respectively. were obtained

A preparative-scale adsorbent column was made by conjugating 2 g of purified anti-I antibody. Low to 110 ml of Sepharose 4B (ref 11) Glycoprotein-pools A and B were dissolved in ammonium acetate buffer (1%. pH 7 5) at a concentration of 15 mg/ml, and enriched for Ii activities by affinity chromatography in 11 batches The yield of enriched glycoprotein was 508 mg (12% of the original material)

Carbohydrate analysis — Monosaccharide compositions were determined by g l c , as described previously[9] Hexose analysis was performed by the phenol–sulphuric acid method[12] using D-galactose as the standard

Degradation with alkaline borohydride of the enriched, sheep glycoproteins in the presence of sodium boro tritide — The degradation was performed by a modification of the method of Iyer and Carlson[7], suggested by Dr. E A Kabat The large quantity of radioactivity used necessitated that the reaction was performed as a tritium-labelling service (TR5) at the Radiochemical Centre (Amersham, Great Britain) To sheep glycoprotein (508 mg) were added distilled water (24 ml), M sodium hydroxide (1 25 ml), sodium borohydride (920 mg), and tritiated sodium borohydride (5 Ci, 5 Ci/mmol), and the solution was heated at 50° for 16 h The mixture was then concentrated to dryness on a rotary evaporator The vessel was cooled with solid carbon dioxide and acetone (6 ml, ~4 molar excess relative to borohydride) was added to decompose residual sodium borohydride* The resulting 2-propanol was evaporated under diminished pressure Acetone (6 ml) was evaporated from the residue, followed by water (20 ml, adjusted to pH 4 with glacial acetic acid) and then methanolic 0 05% acetic acid (10 × 50 ml) The yield of radioactivity was 50 mCi (1%)

*Caution this reaction is potentially explosive

A solution of the residue in distilled water (100 ml) was dialysed[13] in Visking 23/32 tubing (exclusion limit, mol wt 8,000) against 1-litre changes of distilled water, and the diffusible material was collected in three successive fractions. Dial I contained 5 successive dialysates each obtained after 30 min. Dial II contained 3 successive dialysates, each obtained after 24 h, and a fourth obtained after 48 h. Dial III was obtained after dialysis for 96 h. The material remaining in the dialysis tubing after this period was termed non-dial. Dial I and II were combined, and all of the solutions were concentrated under diminished pressure, centrifuged, and lyophilised.

A solution of dial I + II in distilled water was desalted by passage through a column (20 × 1·5 cm) of Dowex AG50 (H^+) resin. Fractions (5 ml) were collected, and those which contained radioactivity were combined and lyophilised. Scintillation counting was performed in a Triton–toluene-based scintillant (liquid scintillation cocktail T, Hopkin and Williams) on an LKB Wallac 8100 liquid scintillation counter.

Gel-permeation chromatography — Bio-Gel P-4 (Biorad Laboratories Ltd.) was packed into two jacketed columns (90 × 2·5 cm) heated at 55° by a Churchill circulator. The columns were connected in series and eluted with distilled water at 18 ml/h, and fractions (1·5 ml) were collected. The columns were calibrated with Blue Dextran (mol. wt., 2 × 10^6, Pharmacia) and an acid hydrolysate[14] of dextran T2000 (Pharmacia). Peaks corresponding to D-glucose oligomers containing 1–16 residues could be resolved (Fig. 2). These peaks were used[15] as calibration points to designate the size of unknown oligosaccharides in terms of hexose units. *N*-acetylhexosamine residues behave as 2 hexose units in this system. Oligosaccharides were detected by the phenol–sulphuric acid reaction or by scintillation counting as described above.

Paper chromatography and high-voltage paper electrophoresis — Whatman No. 40 paper was used. Descending paper chromatography was performed with *A*, 1-butanol–pyridine–water (35:39:26) or *B*, ethyl acetate–pyridine–glacial acetic acid–water (5:5:1:3). High-voltage paper electrophoresis[16] was performed in 0·06M sodium borate buffer (pH 9·5) at 80 V/cm for 2 or 3 h on a Miles Hivolt apparatus (Miles Hivolt Ltd., Shoreham, England). The radioactive materials were located following chromatography with a Packard model 7201 Radiochromatogram scanner.

Antigenic analyses — Blood-group I and i activities were assessed by radioimmunoassays[8] in which glycoproteins or oligosaccharide fractions were used as inhibitors of the binding of anti-I or anti-i antibodies to radio-iodinated I- or Ii-active glycoproteins. Several anti-I sera (Ma, Step, Low, Gra, and Ful) and anti-i sera (Tho, Den, Nic, and Galli) were used, each is known to contain a monoclonal antibody reactive with various domains on a branched, I-active oligosaccharide sequence[1,5,6] or a linear, i-active sequence[4], respectively. In the radio-immunoassays a fraction (termed 20% 2X) of the ovarian-cyst glycoprotein[1,17] OG was used as a reference glycoprotein showing blood-group Ii activity.

Blood-group A and H activities were determined by haemagglutination-

inhibition assays with human anti-A serum and *Ulex europeus* lectin, respectively, as described previously[9]

RESULTS

The activities of the original glycoprotein-pools A and B and of the specifically enriched eluate as inhibitors of the 7 anti-I sera (Table I) are comparable to those of corresponding fractions in analytical experiments described earlier[9] However, pool A differed from pool B and from the previously studied sheep glycoproteins[9] in showing strong blood-group i activity, correspondingly, the specific eluate obtained in this preparative experiment showed very high blood-group i activity The I and i activities of this eluate were substantially higher than those of the reference, Ii-active substance OG (ref. 1)

Moderate blood-group A and H activities were present in the original pools A and B and in the Ii-active eluate (Table I)

Monosaccharide analyses of pools A and B, and of the specific eluate, showed that they contained 72, 70, and 81% of their weight as carbohydrate, and their compositions were similar to corresponding fractions studied previously[9]

TABLE I

BLOOD-GROUP I AND i ACTIVITIES (DETERMINED BY RADIO-IMMUNOASSAYS) AND BLOOD-GROUP A AND H ACTIVITIES (DETERMINED BY HAEMAGGLUTINATION-INHIBITION ASSAYS) OF SHEEP GASTRIC-MUCINS BEFORE AND AFTER AFFINITY CHROMATOGRAPHY ON AN ANTI-I IMMUNOADSORBENT COLUMN

		Pool A	*Pool B*	*Eluate from anti-I column*	*Reference glycoprotein OG*
		Concentration (μg/ml) giving 50% inhibition of binding			
Anti-I	Ma	0 7	0 8	0 1	0 9
	Step	20	28	2	14
	Low	5	10	0 6	5
	Da	7	10	0 7	42
	Gra	4 4	3 8	0 2	5
	Phi	7 5	9 5	0 6	19
	Ver	32	90	3 8	23
Anti-i	Den	12	160	1 7	13
	Tho	19	130	2 7	5 6
	Nic	190	> 1000	24	58
	Galli	46	600	11	28
		Concentration (μg/ml) giving haemagglutination-inhibition			
Anti-A		6	16	12	—[a]
Anti-H		25	12	50	—

[a] —, Previously shown to lack blood-group A and H activities[17]

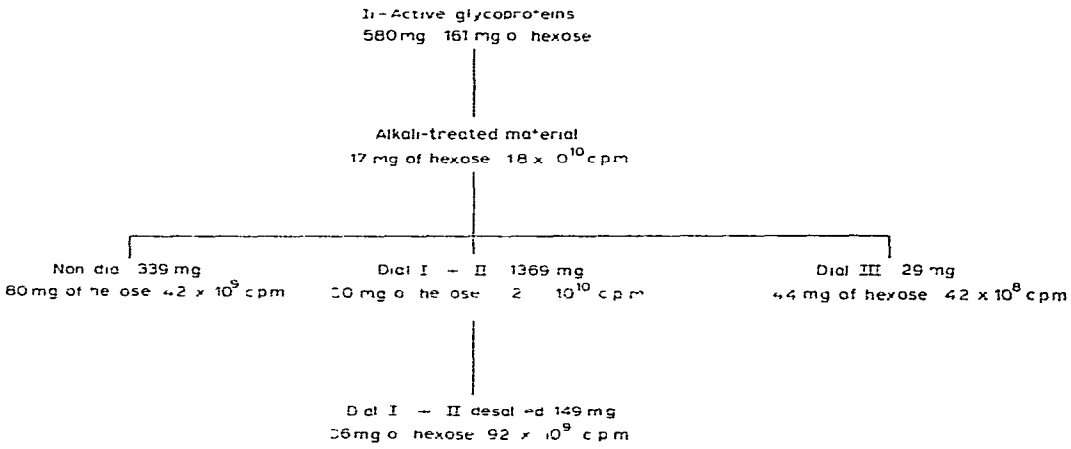


Fig 1 Flow diagram of the alkaline-borohydride degradation of the glycoproteins having blood-group Ii activity and the fractionation of the degraded material by dialysis

Degradation with alkaline borohydride in the presence of sodium borotritide — A flow diagram of the yields of dry weight, hexose, and radioactivity, following the degradation and dialysis procedure, is shown in Fig 1

Two-thirds of the alkali-treated material was not dialysable This non-dialysable fraction was not used in the present studies However, in studies described in detail elsewhere[18], it has been shown to consist of partially degraded glycoproteins with incomplete elimination of the carbohydrate chains and with blood-group Ii activities which were 100–1,000 times lower than those of the undegraded eluate Furthermore, it was shown that the linkages of the residual carbohydrate chains to protein in the non-dial fraction could be cleaved when subjected to a second degradation with alkaline borohydride under the same conditions As expected, there was a lack of GalNAc-ol* in this fraction

Fractionation of dialysable oligosaccharides — Only the dial I + II fraction was used in the present studies for the purification of oligosaccharides Fig 2 shows the radioactive profile of the dial I + II fraction obtained after chromatography on the calibrated column of Bio-Gel P4 21 pooled fractions (A–W) were made as indicated Of each Bio-Gel fraction, ~2 x 10^5 c p m was analysed by paper chromatography (solvent *A*) for 24 or 48 h (results not shown) Increasing migration of the main radioactive peaks in the order A to W indicated that the majority of oligosaccharides had been fractionated according to their molecular weight Fractions A to F showed, in addition, multiple, small, fast-migrating peaks, indicating the presence of some components of low molecular weight in these fractions These findings are in agreement with the work of Yamashita *et al*[15], who showed that charged oligosaccharides (for example, sialylated oligosaccharides) of low molecular

*GalNAc-ol = 2-acetamido-2-deoxy-D-galactitol Except for L-fucose, all of the sugars mentioned in this paper are considered to be in the D series

TABLE II

CARBOHYDRATE COMPOSITION OF THE Bio-Gel-P4 FRACTIONS A, F, K, L, AND S

Fraction	L-*Fuc*	D-*Man*	D-*Glc*	D-*Gal*	D-*GlcNAc*	D-*GalNAc*	D-*GalNAc-ol*	*Sialic acid*	*Estimated number of residues per oligosaccharide*[a]
	(molar ratio of each monosaccharide to D-*GalNAc-ol)*								
A	0.24	0	0	8.82	6.42	0.64	1.00	1.56	18.6
F	0.61	0	0.04	6.34	4.09	0.37	1.00	0	12.4
K	0.49	0	0.37	2.99	3.16	0.60	1.00	0	8.2
L	0.35	0	0.51	2.65	2.78	0.14	1.00	0	6.9
S	0.21	0	0.02	0.96	0.81	0.13	1.00	0	3.1

[a]The number of monosaccharide residues in the oligosaccharide fractions was calculated as the sum of the moles of monosaccharide per mole of D-GalNAc-ol, excluding D-Glc, which was considered a contaminant[9].

weight chromatograph near the void volume of Bio-Gel columns equilibrated in water

Monosaccharide compositions of fractions A, F, K, L, and S — Fractions A, F, K, L, and S were selected for monosaccharide analysis (Table II) Gal and GlcNAc were the main components and they all contained GalNAc-ol Small proportions of L-Fuc and GalNAc were also present As predicted, sialic acid was detected in fraction A The average number of monosaccharide residues per oligosaccharide chain (taken as mol of monosaccharide per mol of GalNAc-ol) in fractions A, F, K, L, and S were 18 6, 12 4, 8 2, 6 9, and 3 1, respectively

Assays of blood-group I and i activities of oligosaccharide fractions — The 21 fractions from the column of Bio-Gel P4 were tested as inhibitors of anti-I sera Ma and Step and anti-i serum Den, certain of the fractions were also tested with anti-I Woj, Low, Gra, and Ful, and anti-i Tho Their inhibitory activities, expressed as μg of hexose giving 50% inhibition of binding, are shown in Fig 3 With fractions A F, K, L, and S, on which monosaccharide-composition data were available (Table II), it was possible to calculate approximately the inhibitory activities in terms of nmoles of oligosaccharides, and these are shown in Table III With fraction R, deductions on its approximate composition could be made from its elution profile on the column of Bio-Gel P4 (see below) The main sub-fraction (LE) of L, obtained after p c (see below), was also tested as an inhibitor of anti-I Ma In Table III the inhibitory

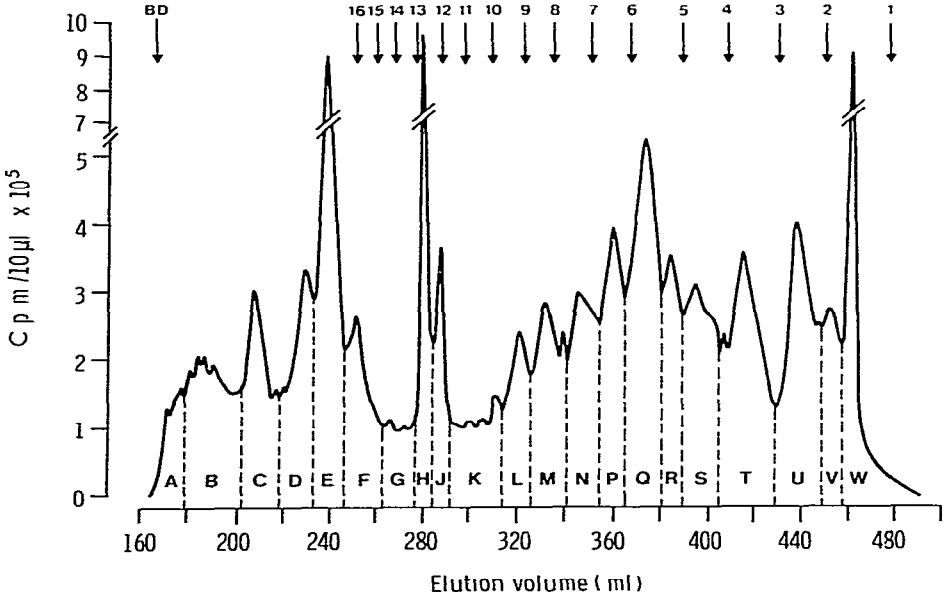


Fig 2 Preparative chromatography on Bio-Gel P4 of the desalted, dial I — II fraction The column was pre-calibrated with the molecular weight markers Blue Dextran (BD) and dextran hydrolysate (glucose oligomers 1–16 arrowed) The distribution of radioactive material is shown by the unbroken line, 21 fractions (A–W) were obtained as shown

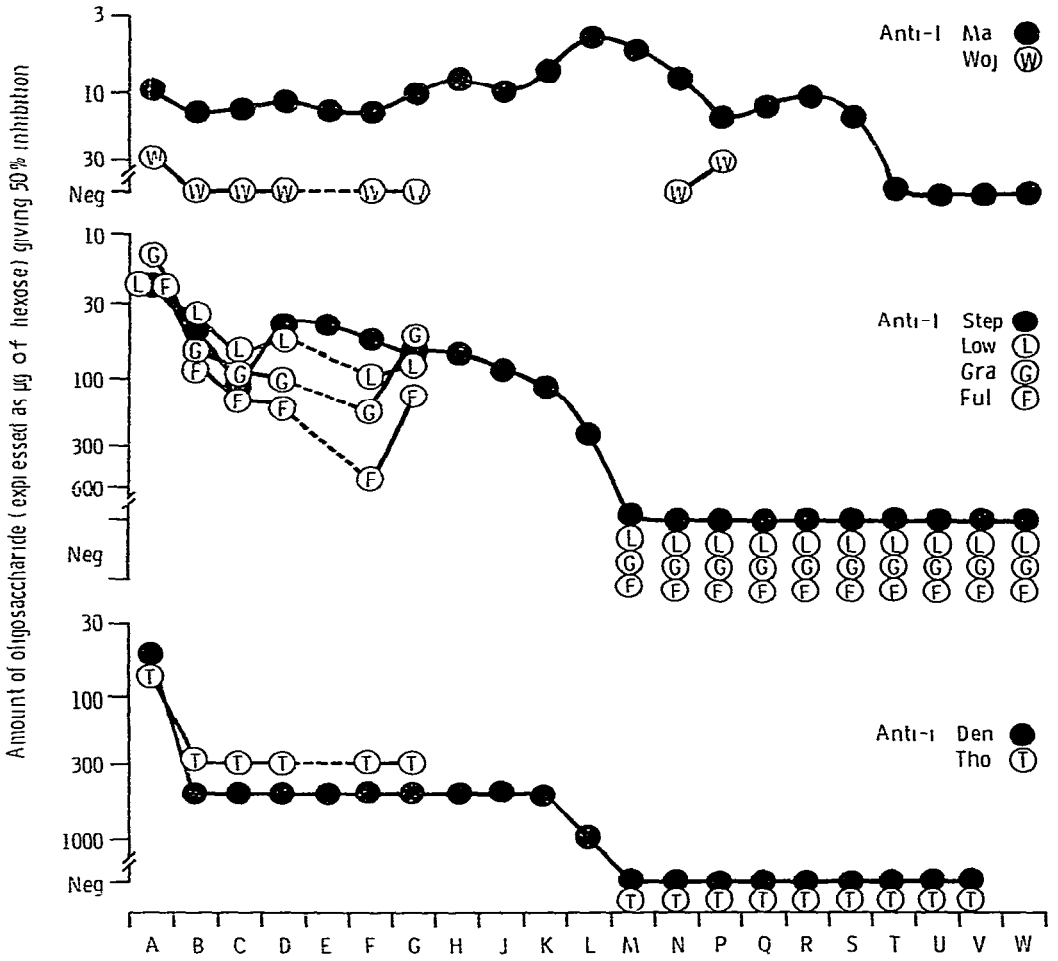


Fig 3 Activities (blood-group I and i) of oligosaccharide fractions A to W obtained by chromatography on Bio-Gel P4 of dialysable oligosaccharides (dial I + II) released by treatment of sheep gastric-mucins with alkaline borohydride All of the fractions were tested by radio-immunoassays as inhibitors of anti-I sera Ma and Step and anti-i serum Den Certain of the fractions were also tested with anti-I Woj, Low, Gra, and Ful, and anti-i Tho

activities of fractions A, F, K, L, LE, R, and S are compared with those of synthetic oligosaccharides (di- to penta-saccharides) previously studied[19]

Inhibitory activities with anti-I sera Ma and Woj, which are known to recognise[1 5 20 21] *the sequence β-Gal-(1→4)-β-GlcNAc-(1→6)* — Fractions A–S showed inhibitory activities with anti-I Ma (Fig 3), those with the smallest molecular weight (fractions S and R) were eluted from Bio-Gel P4 in the region of 4–6 hexose units (Fig 2). Fractions S contained Gal, GlcNAc, and GalNAc-ol in ratios of ~1 1 1 (Table II), in agreement with its elution position on Bio-Gel P4. Fraction R, eluting closer to 6 hexose units, would be predicted to be tetrasaccharide(s) containing these

TABLE III

COMPARISON OF THE AMOUNTS OF OLIGOSACCHARIDE FRACTIONS A, F, K, L, LE AND S AND OF SEVERAL SYNTHETIC OLIGOSACCHARIDES REQUIRED TO GIVE INHIBITION WITH ANTI-I AND ANTI-i SERA IN RADIOIMMUNOASSAYS

Oligosaccharide fractions	*Hexose content per nmol of fraction*[a] *(nmol)*	*(μg)*	*Amount (μg) of hexose (nmol of oligosaccharide) giving 50% inhibition* *Anti-I*		*Anti-i*	
			Ma	*Step*	*Den*	*Tho*
A	8.82	1.59	10 (6)	25 (16)	50 (31)	70 (44)
F	6.34	1.14	15 (13)	55 (48)	500 (439)	300 (263)
K	2.99	0.54	7 (13)	120 (222)	500 (926)	n.t.
L	2.65	0.48	4 (8.3)	220 (458)	1000 (2083)	n.t.
LE	3	0.54	4 (7.4)	n.t.[b]	n.t.	n.t.
R	2	0.36	10 (28)	—[c]	—	—
S	0.96	0.17	15 (88)	—	—	—
Synthetic oligosaccharides[d]						
β-Gal-(1→4)-β-GlcNAc-(1→6)-Gal			(12)	—	—	—
β-Gal-(1→3)-β-GlcNAc-(1 ↘ 3) Gal (6) ↗ β-Gal-(1→4)-β-GlcNAc-(1			(12)	—	—	—
β-Gal-(1→4)-β-GlcNAc-(1 ↘ 3) Gal (6) ↗ β-Gal-(1→4)-β-GlcNAc-(1			(12)	(600)	—	—
β-Gal-(1→4)-β-GlcNAc-(1→3)-Gal			(500)	(1000)	—	(1600)
β-Gal-(1→4)-GlcNAc			(100)	(2400)	n.t.	—

[a]In order to convert the inhibitory activities of the oligosaccharide fractions (determined as μg of hexose giving 50% inhibition) into nmol of oligosaccharide giving inhibition, it was necessary to calculate approximately the hexose contents (mol) per mol of oligosaccharide. These values were calculated from the ratio of Gal to GalNAc-ol in fractions A, F, K, L, and S (given in Table II) or from the proposed composition of fraction R as 2:1:1 and fraction LE as 3:2:1 with respect to Gal/GlcNAc/GalNAc-ol (see Results and ref. 23, respectively). [b]Not tested. [c]Inactive at highest level tested. [d]Taken from refs. 19 and 20.

monosaccharides in ratios of ~2:1:1. The most-active fractions with anti-I Ma (50% inhibition values at 7–8 nmol) were fraction L, which contained hepta- and hexa-saccharides (Table II), and sub-fraction LE, the major component of which has been shown to be a hexasaccharide[22].

Eight of the fractions were also tested with anti-I Woj (Fig. 3). fractions A and P gave 50% inhibition at 30 μg of hexose, but fractions B, C, D, F, G, and N were inactive at the highest level tested (30 μg of hexose).

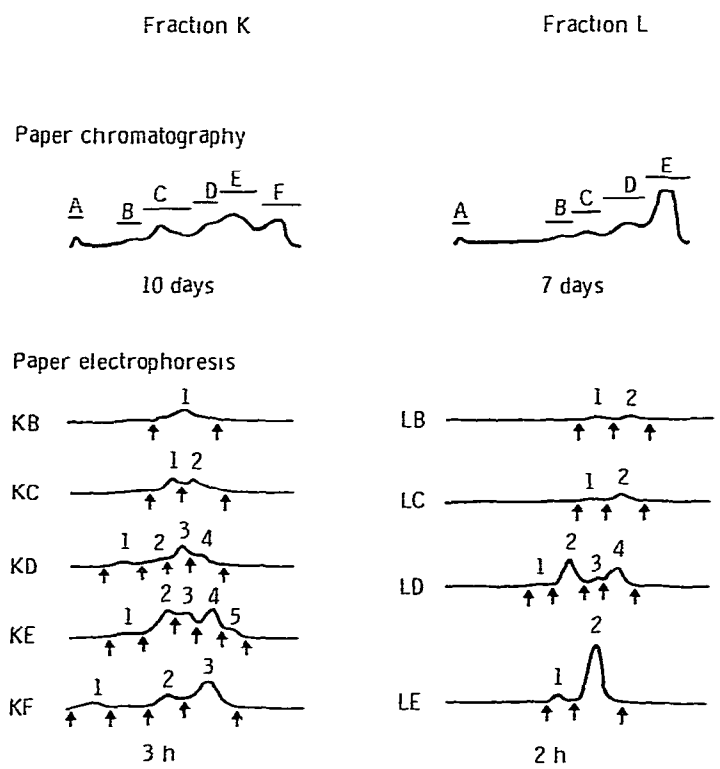


Fig 4 Preparative p c and subsequent high-voltage p e of the preparative, Bio-Gel-P4 fractions K and L Direction of migration is from left to right In p c , the fractions taken are designated by capital letters KA to KF and LA to LE The p e fractions are designated by numbers as shown

Inhibitory activities with anti-I Step and other antisera known to recognise the β-Gal-(1→4)-β-GlcNAc-(1→3) chain of the branched, I-active structure[5 6]. — Inhibitory activities with these anti-I sera were found among oligosaccharide fractions A–L, which were hexasaccharides and larger, with the exception of fraction A, substantially larger amounts of oligosaccharides were required for inhibition (both in terms of hexose and approximate nmol) than with anti-I Ma (Fig 3, Table III)

With anti-I Step, fraction C was a relatively poor inhibitor among the fractions of high molecular weight, otherwise, there was an increase in the inhibitory activities of the fractions L–A with increasing molecular weight The 50%-inhibition values of fractions on which structural or compositional data are available are shown in Table III; they ranged from 16–458 nmol for fractions A–L

Less information is available with anti-I sera Low, Gra, and Ful, but, as with Step, oligosaccharides smaller than hexasaccharides were not active, and the inhibitory activities of the fractions of larger molecular weight tended to increase with increasing molecular weight (Fig 3)

Inhibitory activities with anti-i sera Den and Tho known to react[4] *with the un-*

branched oligosaccharide *β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal* — Inhibitory activities toward these anti-i sera were found among fractions A–L Fraction A was by far the most active inhibitor (Fig 3 and Table III) The 50%-inhibition values of fractions B–K were of the same order with respect to hexose, namely, 500 μg with anti-i Den and 300 μg with those fractions tested with anti-i Tho However, in terms of nmol of oligosaccharides, these inhibition values would represent increasing activities with increasing molecular weight (*e g* 926 and 439 nmol, respectively, for fractions K and F with anti-i Den Table III)

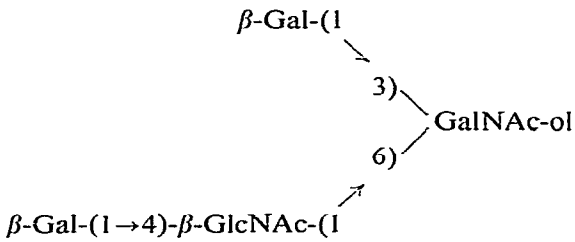
Sub-fractionation of oligosaccharide fractions K and L — The Ii-active fractions obtained in the greatest yield were B, K, and L (2 5 2 5, and 2 mg of hexose respectively) Fractions K and L being the fractions of smallest molecular weight having both I and i activities, were selected for structural studies They were found to consist of mixtures of oligosaccharides They were first subjected to preparative p c (solvent *B*) for 10 and 7 days, respectively, the radioactive profiles are shown in Fig 4. Fraction K was divided into 6 sub-fractions (KA to KF) and fraction L into 5 sub-fractions (LA to LE) The minor fractions KA and LA were not studied further, the remaining sub-fractions of K and L were subjected to p e in borate buffer for 3 h (fractions KB to KF) and 2 h (fractions LB to LE) (Fig 4), 25 sub-fractions were thus obtained and 6 of these (KC_1, KE_2, KF_2 KF_3, LD_2, and LE_2) were selected for further study On the Bio-Gel-P4 column, each fraction was eluted as a single peak, except for KF_3 which gave two peaks (KF_3a and KF_3b) The approximate yields (in μg) of the oligosaccharide fractions were as follows KC_1, 120, KE_2, 200, KF_2 160, KF_3a, 55, KF_3b, 115, LD_2, 80, and LE_2, 600 The structural analyses of these fractions are described in the adjoining paper[23]

DISCUSSION

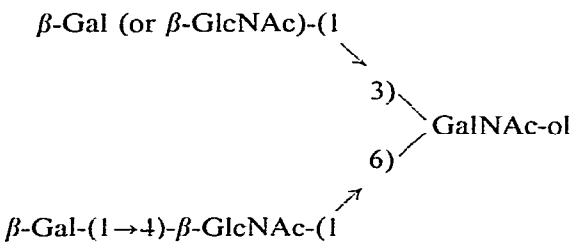
In these studies, a large number of oligosaccharide fractions having blood-group I and i activity were obtained by alkaline degradation of I- and i-active, sheep gastric-glycoproteins that had been specifically enriched by affinity chromatography using an anti-I immunoadsorbent Although the glycoproteins had been selected for high blood-group I activity, only 12% of the macromolecules expressed the various I- and i-antigenic determinants These and previous studies[8 9] with pooled and individual, sheep gastric-mucins indicated that the Ii-active molecules are generally a sub-population even in antigenically very active preparations of protein Without the specific enrichment, the antigenic activities of the oligosaccharide fractions would have been difficult to demonstrate, even by radio-immunoassays

Of the 21 oligosaccharide fractions obtained after chromatography on Bio-Gel P4, 17 showed blood-group i and/or I activities Each fraction was a mixture of oligosaccharides, although the antigenicities of the constituent oligosaccharides were not analysed individually, considerable information could be deduced from the antigenic activities of the fractions Inhibitory activities with anti-I Ma were found in fractions M–S of lower molecular weight in addition to the fractions A–L of higher

molecular weight which were also active with other types of anti-I and anti-i antibodies The smallest of these fractions R and S, were estimated to contain Gal, GlcNAc and GalNAc in ratios of ~2 1 1 and ~1 1 1, respectively The sequence β-Gal-(1→4)-β-GlcNAc-(1→6)- recognised by anti-I Ma is usually not found in glycosphingolipids in the absence of branching[6] It will be of interest to investigate whether the sequence β-Gal-(1→4)-β-GlcNAc-(1→6)-GalNAc-ol exists in fraction S, as the minimum, naturally occurring, "Ma-active" structure in these glycoproteins or whether the antigenic activity of this fraction is carried over from the neighbouring, more-active fraction R, whose composition is compatible with the sequence



Structural data are available for the fraction (L) having most activity with anti-I Ma two of its major components (LE_2 and LD_2) contained the sequence[22 23]

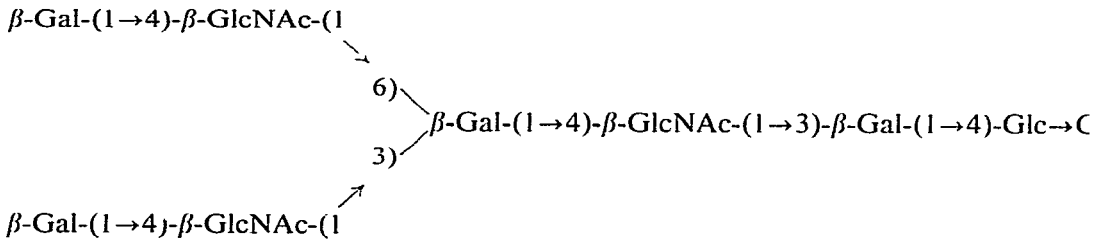


The inhibitory activities of fractions K, L, and (sub-fraction) LE (~13–7 nmol giving 50% inhibition) were of the same order as the activity of synthetic tri- and penta-saccharides containing the sequence β-Gal-(1→4)-β-GlcNAc-(1→6)-Gal 12 nmol of these were required for 50% inhibition (Table III) These data indicate that the antigenic determinant recognised by anti-I Ma is no longer than trisaccharide

With anti-I Woj, several of the oligosaccharide fractions tested were lacking in inhibitory activities or were considerably less active than with anti-I Ma This raises the possibility that some of the (1→4,1→6)-linked chains in these fractions may have external substitutions which mask reactivities with anti-I Woj, but not with anti-I Ma There is a precedent[5 6] for such masking with a bovine-erythrocyte glycosphingolipid containing the sequence α-Gal-(1→3)-β-Gal-(1→4)-β-GlcNAc-(1→6)-

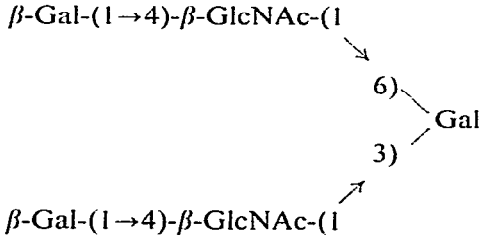
Until recently, the antigenic determinants recognised by anti-I antibodies, other than Ma type, and by anti-i antibodies proved difficult to elucidate. Neither oligosaccharides containing the "type 1" chain-sequence β-Gal-(1→3)-β-GlcNAc-(1→3)-β-Gal-, nor the "type 2" chain-sequence β-Gal-(1→4)-β-GlcNAc-(1→6)-β-Gal-, which are major components in human ovarian-cyst glycoproteins were found

to inhibit these antibodies in inhibition of precipitation assays and in radio-immunoassays[2 20 21] However, it was reported that the type-2 chain-variant β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal- was involved in the antigenic determinant of an anti-i-antibody[24] and detailed antigenic and structural analyses of purified, I- and i-active glycosphingolipids from erythrocytes have clearly established that the specificities of anti-I antibodies (other than those of Ma type) and the majority of anti-i antibodies involve the 1→4,1→3 sequence[4-6] A glycosphingolipid with i-activity had the sequence β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc→Cer, and a related, branched structure with the sequence



had blood-group I activity However, the lack of inhibition with paragloboside[3 4] [β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc→Cer] and the very low activities of the chemically synthesised oligosaccharides[20]

β-Gal-(1→4)-β-GlcNAc-(1→3)-Gal and



suggest that, unlike anti-i Ma, the majority of anti-I and anti-i antibodies may recognise sequences longer than trisaccharide In the present studies, the lack of inhibitory activities among the oligosaccharides smaller than hexasaccharides and the increasing antigenic activities with increasing molecular weight are in support of this concept From the structural analyses in the accompanying paper[23], it is known that fractions K and L contained oligosaccharides having the repeating *N*-acetyl-lactosamine sequence β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal(1→4)-β-GlcNAc However, it is possible that the increasing inhibitory activities are due to the presence of branched, multivalent oligosaccharides

The trisaccharide sequence recognised by anti-I Ma is now well-documented and this antibody has proved useful in detecting accumulation of blood-group precursor in gastric cancer tissues of secretors[25] Future studies with purified hexasaccharides and larger oligosaccharides should enable the size of the antigenic determinants of other, monoclonal, anti-I and anti-i antibodies to be defined Such information will render these autoantibodies powerful tools in biological chemistry

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