BLOOD GROUP I AND i ACTIVITIES OF STRAIGHT CHAIN AND BRANCHED SYNTHETIC OLIGOSACCHARIDES RELATED TO THE PRECURSORS OF THE MAJOR BLOOD GROUP ANTIGENS

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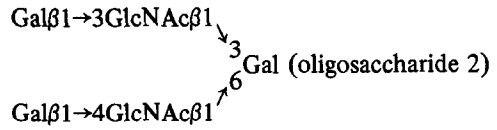
1. Introduction

Advances in synthetic carbohydrate chemistry have resulted in the chemical synthesis of oligosaccharide chains related to the precursors of the major blood group antigens, and such synthetic oligosaccharides were recently shown to be of value in defining the specificities of anti-I antibodies [1,2]. The synthetic oligosaccharides:

Galβ1→4GlcNAcβ1→6Gal (oligosaccharide 4)

and



were shown to be indistinguishable from authentic oligosaccharides containing the unsubstituted sequence Galβ1→4GlcNAcβ1→6Gal in their inhibitory activities with the anti-I antibody of patient Ma [1]. Radioimmunoassays with 10 other anti-I sera showed that a second anti-I antibody (from patient Woj) was also inhibited by oligosaccharides 2 and 4. None of the anti-I sera were inhibited by the synthetic oligosaccharide analogues:

Galβ1→3GlcNAcβ1→3Gal (oligosaccharide 3)

Galβ1→3GlcNAcβ1→6Gal (oligosaccharide 5)

Abbreviations: Gal, *D*-galactopyranose; GlcNAc, 2-acetamido 2-deoxy-D-glucopyranose; Cer, ceramide

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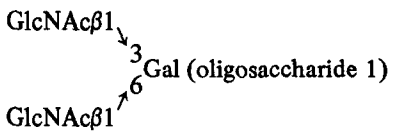
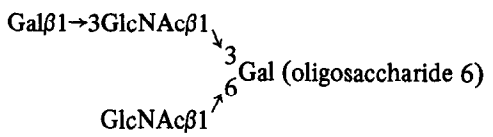
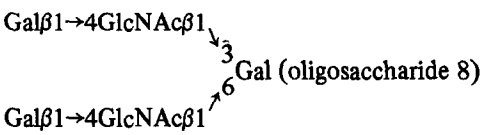
and



at the highest levels tested (108–182 nmol). In the present studies the I and i activities of additional synthetic oligosaccharides:



Galβ1→4GlcNAcβ1→3Gal (oligosaccharide 7)



Galβ1→4GlcNAc (oligosaccharide 9)

and Galβ1→3GlcNAc (oligosaccharide 10) have been determined by radioimmunoassays and it has been shown that oligosaccharides containing the sequence Galβ1→4GlcNAcβ1→3Gal have inhibitory activity toward the majority of anti-I (other than Ma and Woj) and anti-i antibodies. However, the amounts of these 1→4, 1→3 linked oligosaccharides required to demonstrate inhibition were 50–100-times greater, on a molar basis, than the amounts of 1→4, 1→6 linked oligosaccharides required with anti-I Ma and

Table 1
Synthetic oligosaccharides used as inhibitors with 6 anti-I and 3 anti-i sera in radioimmunoassays

Oligosaccharide (designation)	Ref.	Anti-I						Anti-i		
		Ma[a] 1:4000	Woj 1:500	Step 1:1600	Da 1:1600	Ver 1:2000	Zg 1:600	McC 1:15,000	Tho 1:1600	Den 1:80,000
		Maximum amount of oligosaccharide tested (nmol)								
GlcNAcβ1 ↘ 3 Gal (1) ↗ 6 GlcNAcβ1	[7]	170[b]	170[b]	1530	1020	1020	1530	1020	1020	170[b]
Galβ1→3GlcNAcβ1 ↘ 3 Gal (2) ↗ 6 Galβ1→4GlcNAcβ1	[8]	66[b]	66[b]	990	990	990	n.t.[c]	106[b]	106[b]	106[b]
Galβ1→3GlcNAcβ1→3Gal (3)	[9]	183[b]	108[b]	183[b]	183[b]	183[b]	n.t.	183[b]	183[b]	183[b]
Galβ1→4GlcNAcβ1→6Gal (4)	[10]	183[b]	183[b]	1647	1098	1647	n.t.	1098	824	183[b]
Galβ1→3GlcNAcβ1→6Gal (5)	[10]	549[b]	549[b]	183[b]	183[b]	183[b]	n.t.	183[b]	183[b]	183[b]
Galβ1→3GlcNAcβ1 ↘ 3 Gal (6) ↗ 6 GlcNAcβ1	[10]	402	402	804	804	402	1206	402	402	402
Galβ1→4GlcNAcβ1→3Gal (7)	[10]	549	549	1647	1098	1647	1647	1647	1647	549
Galβ1→4GlcNAcβ1 ↘ 3 Gal (8) ↗ 6 Galβ1→4GlcNAcβ1	[10]	33	33	990	495	990	n.t.	990	990	n.t.
Galβ1→4GlcNAc (9)	[10]	783	783	2349	2349	2349	2349	2349	783	783
Galβ1→3GlcNAc (10)	Commercial	240	240	2349	n.t.	2349	n.t.	2349	n.t.	n.t.
Galβ1→4Glc (L)	Commercial	260	260	2628	n.t.	2628	n.t.	2628	n.t.	n.t.

[a] Radioimmunoassays with the anti-I sera Ma, Woj, Step, Da and Ver were performed with a radioiodinated blood group I-active sheep glycoprotein and the remaining antisera with a radioiodinated blood group Ii-active human glycoprotein [11]

[b] Tests carried out in [1]

[c] n.t., not tested

Woj. These observations support earlier suggestions [3] that for optimal reactivity the binding sites of the former antibodies require additional antigenic determinants on longer oligosaccharide chains.

2. Materials and methods

2.1. *Anti-I and anti-i sera*

Six anti-I sera (Ma, Woj, Step, Da, Ver, Zg) and three anti-i sera (McC, Tho, Den) were studied; they were all obtained from patients with chronic cold agglutinin syndrome and have been described [1,3–6].

2.2. *Oligosaccharides*

The oligosaccharides used as inhibitors of the anti-I and anti-i sera in radioimmunoassays are shown in table 1. Oligosaccharides 1–9 were prepared by chemical synthesis [7–10] in the laboratory of Professor S. David. Oligosaccharide 10 and lactose (L) were purchased, respectively, from Sefochemical Fine Chemicals Ltd., Israel, and Sigma London Chemical Co., England. The numerical designation for the synthetic oligosaccharides was empirical: designations 1–5 were used as in [1].

2.3. *Radioimmunoassays*

The inhibitory activities of the oligosaccharides with the anti-I and anti-i sera were evaluated by a double antibody radioimmunoassay as in [1,11]. The results were expressed as nmol oligosaccharides required to give 50% inhibition of binding of the antisera to ^{125}I-labelled blood group I or Ii active glycoproteins. Table 1 shows the maximum amount of each oligosaccharide tested in the present and the previous [1] studies.

3. Results and discussion

The specificity of the anti-I antibodies Ma and Woj for the sequence Galβ1→4GlcNAcβ1→6Gal was further substantiated, for oligosaccharide 8 resembled oligosaccharides 2 and 4 in giving 50% inhibition of binding at 12 and 16 nmol, respectively, with these two antisera (fig.1). Oligosaccharides 7 and 9 which contain only a part of this sequence (Galβ1→4GlcNAc) were considerably less active and 100–500 nmol were

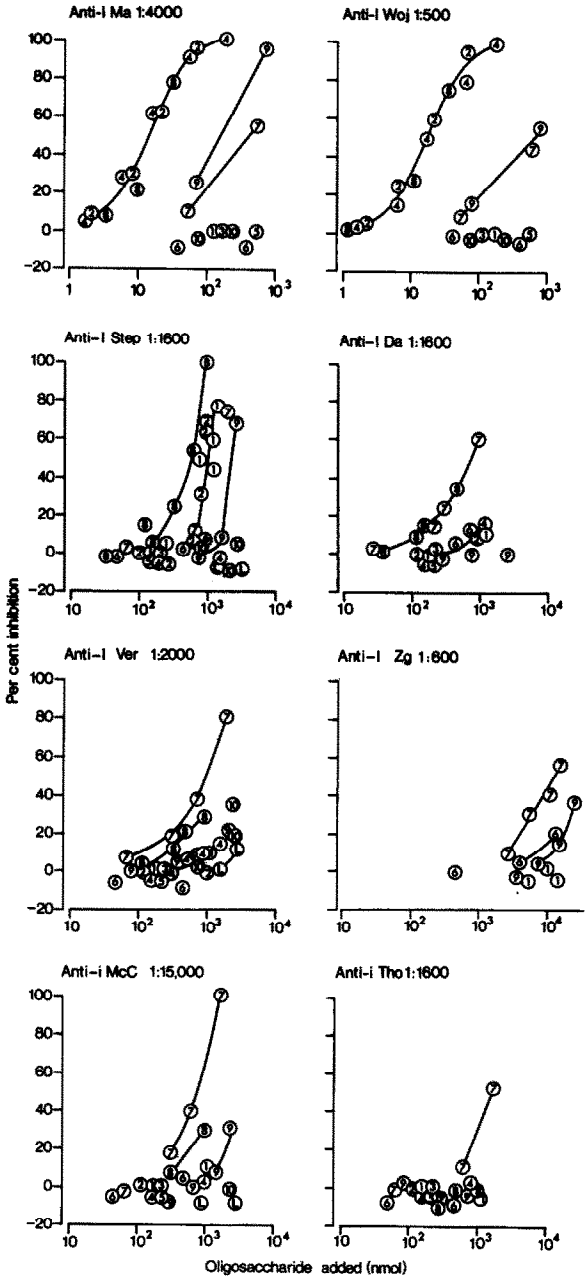


Fig.1 Inhibition of binding of 6 anti-I and 2 anti-i sera to ^{125}I-labelled blood group I-active or Ii-active glycoproteins by synthetic oligosaccharide. Symbols: 1–10 and L refer to oligosaccharides as defined in table 1. A third anti-i serum Den showed no inhibition with oligosaccharides 1–7 and 9 at the levels indicated in table 1 and is not illustrated. For completeness the radioimmunoassay data from an earlier study [1] are also included.

Table 2
Comparison of the amounts of free oligosaccharides and glycolipids inserted in cholesterol/lecithin liposomes required to give inhibition with anti-I and anti-i sera in radioimmunoassays

Inhibitors	Anti-I						Anti-i	
	Ma	Woj	Step	Da	Ver	Zg	McC	Tho
	nmol giving 50% inhibition							
Galβ1→4GlcNAc (oligosaccharide 9)	100	500	2400	–[a]	(10%)[b]	(10%) ≃3200[c]	– ≃4000	–
Galβ1→4GlcNAcβ1→6Gal (oligosaccharide 4)	12	16	–	(12%)	(10%)	n.t.[f]	–	–
Galβ1→4GlcNAcβ1→3Gal (oligosaccharide 7)	500	500	1000	700	1000	1200	800	1600
Galβ1→4GlcNAcβ1 ↘ 6 3 Gal ↗ Galβ1→4GlcNAcβ1 (oligosaccharide 8)	12	16	600	700	(30%) ≃2000	n.t.	(30%) ≃2000	–
Galβ1→4GlcNAcβ1→6Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ→Cer (structure VII)[d]	0.1	0.2	–[e]	–	–	–	–	–
Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ→Cer (structure VIII)[d]	–	–	0.3	–	0.03	–	0.08	0.02
Galβ1→4GlcNAcβ1 ↘ 6 3 Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ→Cer (structure III)[d] ↗ Galβ1→4GlcNAcβ1	0.2	0.2	0.02	0.05	0.03	–	–	–

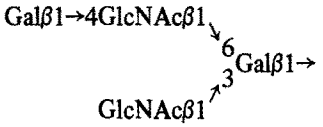
[a] With the oligosaccharides; – = no inhibition detected at 1000 nmol
[b] The figures in parentheses indicate % inhibition at 1000 nm
[c] The figures preceded by ≃ are an estimate of nmol oligosaccharide required for 50% inhibition obtained by reasonable extrapolation
[d] The data shown for the glycosphingolipid structures III, VII and VIII are derived from [4,5]
[e] With the glycosphingolipids; – = no inhibition at 0.3 nmol
[f] n.t., not tested

required for 50% inhibition. Oligosaccharides 1,3,5 and 10 which lacked this sequence were not active.

Inhibition of the anti-I sera Step, Da, Ver, Zg and the anti-i sera McC and Tho was obtained with oligosaccharides 7 or 8 which contain the sequence Galβ1→4GlcNAcβ1→3Gal. The reactivity of the anti-i antibody McC with the milk oligosaccharide lacto-*N*-neotetraose which contains the 1→4, 1→3 sequence has been reported [6]. However, the amounts of these oligosaccharides required to give 50% inhibition were very high, ranging from 600 to ~ 2000 nmol (fig.1, table 2). In agreement with previous inhibition data with purified glycosphingolipids derived from erythrocytes [4,5], oligosaccharide 8 (containing the 1→4, 1→3 sequence as part of a branched structure) was more active than the straight chain oligosaccharide 7 with anti-I Step, and only the latter oligosaccharide was active with anti-i Tho. However, with anti-I Da, the two oligosaccharides were equally active, and with anti-I Ver and anti-i McC oligosaccharide 7 was slightly more active than 8. In exploratory experiments with anti-i Den, no inhibition was observed (table 1, fig.1); this antiserum was not investigated further.

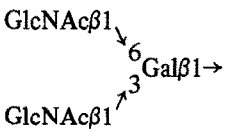
In table 2 a comparison is made of the amounts of oligosaccharides 4, 7, 8 and 9 required in the present studies and the amounts of the purified I and i active glycosphingolipids, structures III, VII and VIII [4,5], that were previously required to inhibit these antisera. Since the glycosphingolipids were assayed after polymerization on cholesterol/lecithin liposomes, far smaller amounts of them were required (0.02–0.3 nmol) than the oligosaccharides to give 50% inhibition of binding (these amounts, 20–300 ng inhibitor, are comparable to the amounts of Ii-active complex glycoproteins usually required to give comparable inhibition [1,5]). In contrast to the requirements with the oligosaccharides in the present studies, the amounts of the glycosphingolipid structures III and/or VIII required to inhibit anti-I sera Step, Da and Ver and anti-i McC and Tho were not greater than the amounts of structures III or VII that were required to inhibit Ma and Woj. In fact the amounts of structure VIII required with the anti-i sera McC and Tho were substantially smaller. These latter antisera may require 50–100-fold greater amounts of oligosaccharides 7 and 8 because these oligosaccharides represent only a part of the antigenic determinants they recognize. These observations are in support of previous suggestions that the antigens recognized by anti-i antibodies involve oligosaccharide chains with a repeating Galβ1→4GlcNAcβ1→3 sequence [3].

It is of interest that oligosaccharides 1, 2 and 7 were equally active as inhibitors of anti-I Step, suggesting that the -GlcNAcβ1→3Gal- component of the Galβ1→4GlcNAcβ1→3Gal-sequence contains important antigenic determinants reactive with the combining site of this antibody. The glycosphingolipid:



4GlcNAcβ1→3Galβ1→4Glcβ→Cer (structure IV)

was previously shown to be inhibitory with this antiserum at ~0.3 nmol [4]. However, its analogue, devoid of the terminal galactose:



4GlcNAcβ1→3Galβ1→4Glcβ→Cer (structure VI)

was not active at 0.3 nmol. It is tempting to speculate that this discrepancy may be due to a difference in the conformation of a free oligosaccharide as compared with the same sequence on a glycosphingolipid inserted into a liposome. However, it would be necessary to test the glycosphingolipids at levels > 0.3 nmol to confirm their lack of activity.

The significance of the weak inhibitions given by certain of the oligosaccharides at levels > 1000 nmol (fig.1) is uncertain. The total lack of inhibition with other oligosaccharides at this level suggests that the weak inhibitions observed may be due to a partial recognition of these structures.

Since anti-I Zg differed from 10 other anti-I antibodies in not being inhibited (at the highest dose tested, 0.3 nmol) by the glycosphingolipid structures III, VII or VIII which contain branched or straight 'type 2' oligosaccharide chains with terminal Galβ1→4GlcNAc sequence ([5], table 2), it was of interest to evaluate its reactivity with 'type 1'

oligosaccharide chains with terminal Galβ1→3Gal sequence. Limited amounts of oligosaccharides were available for testing with this antiserum (table 1, fig.1). However, there was no evidence for preferential reactivity with 'type 1' chains; on the contrary, oligosaccharide 7 with 1→4, 1→3 sequence, was a considerably better inhibitor than oligosaccharide 6 which contains the 1→3, 1→3 sequence.

From these data it can be anticipated that antigenic analysis with longer synthetic oligosaccharide analogues will contribute substantially to the definition of the antigenic determinants recognized by various monoclonal anti-I and anti-i antibodies for they will provide opportunities to investigate the antigenic roles of internal residues on blood group precursor chains. Thus they will usefully supplement the information already available from purified glycosphingolipids of erythrocytes.

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