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Carbohydrate Research 308 (1998) 191–193

Note

Synthetic C-oligosaccharides mimic their natural, analogous immunodeterminants in binding to three monoclonal immunoglobulins

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Received 12 January 1998; accepted 27 February 1998

Abstract

The binding of three monoclonal antigalactan immunoglobulins, IgAs X24, J539 and X44 to their natural haptens methyl β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside, and the corresponding tri- and tetrasaccharides, was compared to the binding of these immunoglobulins with the comparable C-linked oligosaccharide analogues **1**–**3**. The near identity of affinities of the two sets of oligosaccharides indicated the absence of any hydrogen bond involvement by the intersaccharidic oxygen atoms in the natural immunodeterminants. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: Monoclonal immunoglobulins; C-oligosaccharides; Binding

We have reported extensively on the binding of anti-galactan, monoclonal immunoglobulins with *O*-β-(1→6)-linked D-galactopyranosyl-containing oligosaccharides, including their binding with derivatized saccharides so as to elucidate hydrogen bond interactions [1]. The intersaccharidic oxygen atoms of these oligosaccharides were hitherto not probed for their capability to receive hydrogen bonds from the protein. For that reason we have used three monoclonal immunoglobulins specific for the naturally occuring *O*-β-(1→6)-linked D-galactopyranosyl sequence and have measured their binding to corresponding oligosaccharides having their intersaccharidic O atoms replaced by a methylene group, i.e., the C-oligosaccharide analogues. Synthetic C-disaccharides were first reported by Rouzaud and Sinaÿ [2]. C-oligosaccharides are impervious to aqueous hydrolytic fragmentation, chemically or biologically. As such they can be important for use as immunogens (possibly linked to a protein carrier) or as markers linked to drugs in order to direct their delivery to specific receptors on cell surfaces. It is therefore of interest to elucidate the affinity of these carba-analogues for immunoglobulins specific for the naturally occuring immunodeterminant oligosaccharides.

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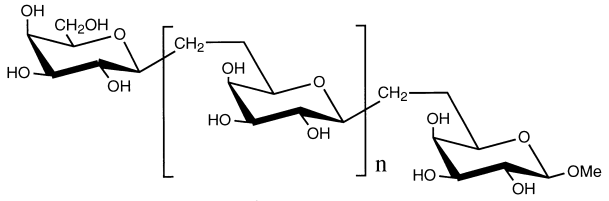
0008-6215/98/$19.00
PII S0008-6215(98)00071-8

The conformational flexibility of the (1→6)-hexopyranosyl linkage makes the corresponding C-oligosaccharide analogues likely to easily assume a conformation similar to that of the corresponding O-analogues, and experimental support for the conformational similarity between C- and O-linked disaccharides has indeed been shown [3–5].

1. Results and discussion



1 n = 0
2 n = 1
3 n = 2

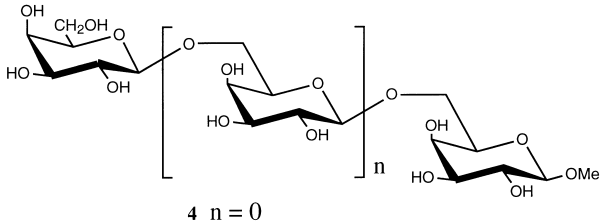
The preparation of the following methyl β-glycosidic C-oligosaccharides here used will be reported separately: methyl 6-deoxy-6-*C*-(2,6-anhydro-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-β-D-galactopyranoside (**1**) [mp 203 °C, $[\alpha]_D$ −10° (*c* 1, methanol)]; methyl 6-deoxy-6-*C*-[2,6-anhydro-6-*C*-(2,6-anhydro-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-β-D-*glycero*-L-*manno*-heptitol-1-yl]-β-D-galactopyranoside (**2**) [mp 187 °C (dec.), $[\alpha]_D$ −4° (*c* 0.2, methanol)]; and methyl 6-deoxy-6-*C*-{2,6-anhydro-6-deoxy-6-*C*-[2,6-anhydro-6-deoxy-6-*C*-(2,6-anhydro-β-D-*glycero*-L-*manno*-heptitol-1-yl)-β-D-*glycero*-L-*manno*-heptitol-1-yl]-β-D-*glycero*-L-*manno*-heptitol-1-yl}-β-D-galactopyranoside (**3**) [mp 154 °C (dec.), $[\alpha]_D$ −4° (*c* 1, 3:2 methanol–water)]. The three corresponding O-linked methyl glycosides: methyl β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**4**), methyl β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**5**), and methyl β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**6**), have been described previously [6].

The binding of the synthetic C-oligosaccharides **1**, **2** and **3** to three anti-galactan monoclonal immunoglobulins was compared to that of the O-linked oligosaccharides **4**, **5** and **6** that constitute the natural immunodeterminants for these three immunoglobulins [1,7]. The results are listed in Table 1. The use of oligosaccharides in which the downstream [8] terminal sugar residue has been converted to the methyl glycoside allows the interpretation of binding data to be free of the ambiguities that would otherwise result from various ring- and open-chain equilibria expected if the terminal sugar were a reducing one.

Table 1
Comparison of the free energy of association, $-\Delta G^\circ$, and maximum percentage change in ligand-induced fluorescence (ΔF_{max}) of O- and C-oligosaccharides with three anti-galactan immunoglobulins [a]

Compound	$-\Delta G^\circ$ in kJ/mol (ΔF_{max} %)		
	IgA X-44	IgA J539	IgA X-24
4	23.6 (−8)	26.7 (+41)	24.7 (+35)
1	19.8 (−13)	25.6 (+36)	24.0 (+26)
5	30.4 (−11)	32.4 (+43)	31.4 (+41)
2	30.7 (−7)	30.7 (+40)	30.4 (+39)
6	31.4 (−9)	32.9 (+41)	32.8 (+40)
3	30.1 (−7)	31.0 (+39)	30.4 (+35)

[a] The affinity constants for **4**, **5** and **6** were previously reported [7]. The affinity constants here measured with each of the three IgAs and the three C-oligosaccharides **1**, **2** and **3**, each had Scatchard plots having a minimum of seven points. These ranged over a wide spectrum of values for fractional site-occupation (ν). Their correlation factors (R^2) ranged from 0.949 to 0.992, except for the plot of **3** and IgA X-44, which had a correlation factor of 0.856.



4 n = 0
5 n = 1
6 n = 2

The three immunoglobulins selected for our binding studies all belong to the group of anti-O-β-(1→6)-D-galactopyranan antibodies [7]. Their idiotypi, as well as their solution-binding to ligands, and their H,L heterologous chain recombinations have been extensively studied [1]. One of them, IgA X24, constitutes the prototype of this group of immunoglobulins [9] that also includes IgAs J539 and X44. The latter IgA was included because it shows a quenched ligand-induced fluorescence behaviour on binding with the natural immunodeterminants, while the other immunoglobulins in this group show an enhanced fluorescence upon binding the natural immunodeterminants. This is most likely due to the fact that IgA X44 is the only IgA in this group that, instead of an isoleucine residue, possesses a tryptophanyl residue at position

96L. The latter is capable of stacking with the tryptophanyl residue at position 91L, thereby altering the ligand-induced fluorescence behaviour of that antibody in a unique fashion [7]. This property can therefore help to 'fingerprint' the nature of the binding of ligands in the immunoglobulin's combining area at the H/L chain interface.

It can be seen in Table 1 that the free energies of association for each C-oligosaccharide with each of the three immunoglobulins are very close to those of the corresponding natural O-oligosaccharides with these same immunoglobulins. The maximum percentage change of fluorescence of the protein induced by both sets of ligands is also similar and of the same sign, i.e., the unique quenching, rather than the enhancement, of the natural ligand-induced tryptophanyl fluorescence change of IgA X44 is paralleled by the C-oligosaccharides. The above shows that the nature of the binding of these two classes of saccharides to the immunoglobulins appears essentially identical. Thus, we have presented evidence that the intersaccharidic oxygen atoms in any of the oligosaccharides **4**, **5**, or **6** do not partake in critical polar interactions, including hydrogen bonding, with the binding area of these immunoglobulins. In addition, this work shows that C-linked oligosaccharides, analogous to natural immunodeterminants, can bind to highly specific biological receptors such as immunoglobulins in the same manner as do the natural ligands. Others have investigated the binding of certain C-saccharides to proteins [5,10–12]. In many cases these involve comparisons of O- and C-saccharides that differ in addition to the substitution –O– → –CH_2–, and therefore these comparisons might be flawed. In one case however [5], the comparisons are all between the lectin-binding of oligosaccharides whose differences exist only in the glycosidic oxygen/methylene. There, near identity of binding was found, as we do in the case of the galactopyranosyl oligosaccharides and immunoglobulins reported here.

It should be remembered that the identical, or nearly identical, affinity constants do not imply that the kinetics must be identical also. However, the great similarity of ligand-induced tryptophanyl fluorescence change, as well as affinity, shown by the sets of corresponding ligands when binding to the immunoglobulins strongly suggests near identity in binding for the natural O- and C-oligosaccharides.

2. Experimental

Immunoglobulins were purified by affinity chromatography as described previously [13]. Ligand-induced fluorescence changes of the three immunoglobulins due to binding each of the three C-oligosaccharides were measured as described before [14,15]. For the equilibrium IgA_{site} + ligand$\rightleftarrows IgA_{site}$—ligand, the expression $K_a = \nu/(1-\nu)c_L$ was used, where K_a is the affinity constant, ν is the fraction of antibody sites bound to ligand, $(1-\nu)$ the fraction of free antibody sites, and c_L is the concentration of free ligand. Thus, a Scatchard plot of ν/c_L versus ν gives a line whose slope is equal to K_a. The free energy, $-\Delta G^\circ$, in kJ/mole, was then obtained from $-\Delta G^\circ = RT \ln K_a$. These values and the maximum ligand-induced fluorescence changes (in %) are listed in Table 1.

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