O-Linked oligosaccharides from human serum immunoglobulin A1

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Introduction

The glycosylation of normal human serum immunoglobulins (Igs) has only been elucidated in detail for IgG [1]. In the case of other Igs, e.g. IgA, myeloma proteins have been used to study the carbohydrate moieties [2], but as these glycoproteins may be abnormally glycosylated they may poorly reflect the carbohydrate of normal serum Igs [3]. As part of an investigation into the glycosylation of normal human serum IgA and IgA from patients with various autoimmune disorders, the structures of the *O*-linked oligosaccharides from IgA1 (located at the hinge region of the molecule) have been determined.

Protocol

Three milligrams of monomeric human serum IgA1 were purified from pooled normal serum by affinity chromatography on immobilized jacalin lectin (which binds the T-antigen, Galβ1 → 3GalNAc) followed by h.p.l.c. gel filtration [4]. The *O*-linked oligosaccharides were released by β-elimination in the presence of sodium [^{3}H]borohydride, and purified by the method of Amano *et al.* [5]. The oligosaccharides were then fractionated by anion exchange on Mono-Q and gel filtration on Bio-Gel P4. The oligosaccharides were subjected to structural analysis by a combination of gas chromatography–mass spectrometry (g.c.–m.s.) methylation analysis, enzymic degradation and

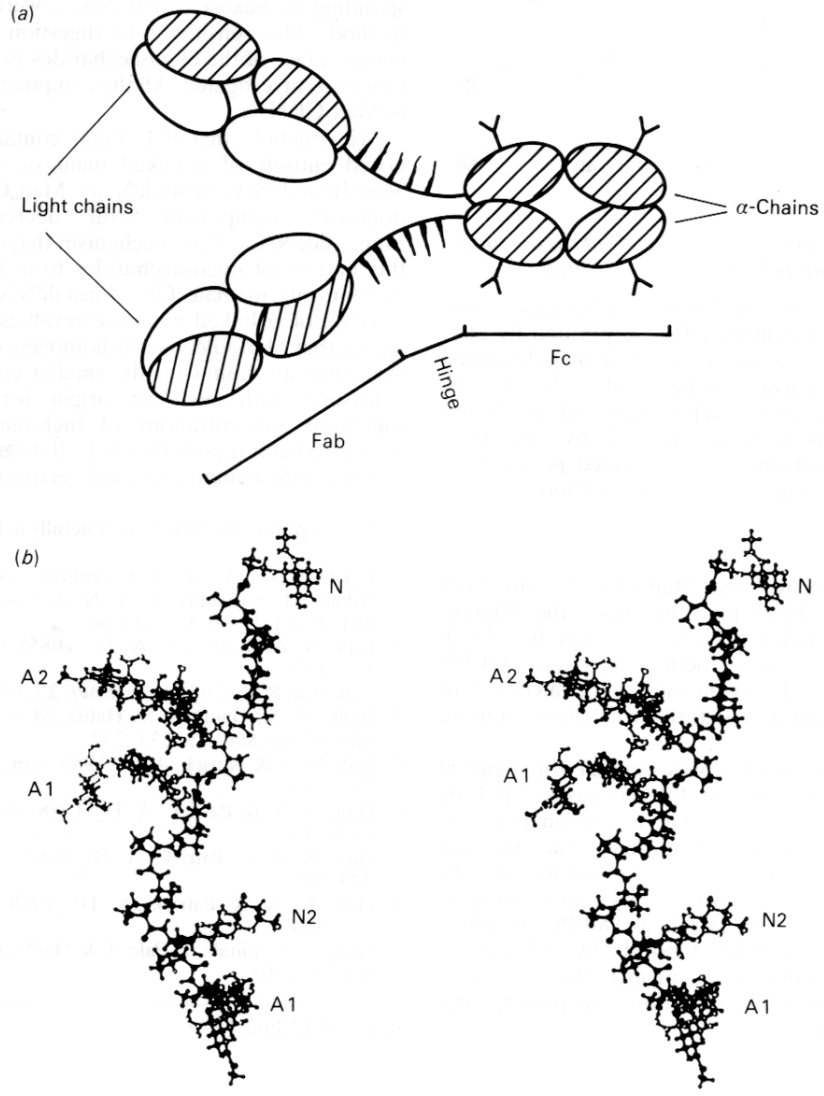

Fig. 1. *(a) Human serum immunoglobulin A1 and (b) stereo diagram showing the relative sizes of the polypeptide and carbohydrate in the hinge region of human serum IgA1*

(*a*) The heavy chains are shown shaded. The positions of *N*-linked oligosaccharides are indicated by 'Y', and the *O*-linked oligosaccharides by bars in the hinge region. (*b*) The lettering indicates the glycans as designated in Table 1. The distribution of the sialic acids among the glycans is arbitrary.

g.c.–m.s. composition analysis. For the generation of Fig. 1(*b*), the hinge region polypeptide, sequence STPPTPSPSTPPTPSPS [2], was assembled and minimized using BIOSYM software [6]. The carbohydrates (N1, N2, A1 and A2, Table 1) were then added to the appropriate serines in reasonable solution conformations as determined from n.m.r. studies of similar compounds.

Results

Three oligosaccharide fractions were resolved by anion exchange, a neutral component (N, 36.0%), and two acidic components (A1, 54.5% and A2, 9.5%). The latter eluted from the anion-exchange column with the same retention times as authentic monosialyl- and disialyl-oligosaccharides, respectively. Methylation analysis of A1 and A2 identified 3-linked galactose and 3-linked *N*-acetylgalactosaminitol (A1), and 3-linked galactose and 3,6-linked *N*-acetylgalactosaminitol (A2). Analysis of per-trimethylsilyl glycosides by g.c.–m.s. after mild acid treatment [7] identified *N*-acetylneuraminic acid as the only sialic acid present in A1 and A2.

Digestion with *Artherobacter ureafaciens* neuraminidase converted all the oligosaccharides to neutral. Bio-Gel P4 chromatography identified two components in the neutral (N) fraction, eluting at 3.5 glucose units (gu), (N2, 48.7%) and 2.5 gu (N1, 51.3%), while in the acidic fractions following desialylation only a 3.5 gu of glycan was observed. All the 3.5 gu oligosaccharides eluted at 2.5 gu from Bio-Gel P4 following digestion with bovine epididymal β-galactosidase. The naturally occurring 2.5 gu saccharide migrated on borate high-voltage electrophoresis to a position consistent with *N*-acetylgalactosaminitol. Based on these data, the structures in Table 1 are proposed for the *O*-linked oligosaccharides of human serum IgA1. The molar incidence of each saccharide was calculated from quantification of the radiolabel in the fractions obtained by anion-exchange chromatography and of the incidence of the 2.5 and 3.5 gu glycans resolved on Bio-Gel P4 obtained from the naturally neutral oligosaccharides.

Discussion

The structures presented here are different from those of *O*-linked oligosaccharides found on a human myeloma IgA1 [2], in that sialylated *O*-linked glycans (in total 64%) were found. However, the ratio of monosaccharide to disaccharide neutral cores (1:4) is the same in both studies. The presence of sialylated oligosaccharides as part of the hinge region of IgA1 may have important physicochemical consequences since the hinge region polypeptide is devoid of charged amino acids [8]. In addition, it seems quite likely that the four oligosaccharides are not distributed in a uniform manner throughout the population of IgA1 molecules and therefore could give rise to IgA1 glycoforms based on differential *O*-linked glycosylation. Because IgA1 has been shown to bind to a macrophage α-chain Fc receptor by virtue of the hinge region β-galactose residues [9], the distribution of sialic acids on these glycans may alter the affinity of IgA1 glycoforms to interact with Fc receptors and therefore generate a spectrum of IgA1 molecules with subtle differences in function. Fig. 1 illustrates the relative sizes of the oligosaccharides and the hinge peptide in the IgA1 molecule. The oligosaccharide moieties account for a large proportion of the exposed surface area of the IgA1 hinge region (Fig. 1*b*). The steric effects of the oligosaccharides on each other could be important in modulation of events involving recognition of the hinge region peptide or carbohydrate.

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Table 1. *Proposed structures for the O-linked oligosaccharides of human serum IgA1*

All residues are in the pyranose D-configuration. $GalNAc_{OL}$, *N*-acetylgalactosaminitol; NeuNAc, *N*-acetylneuraminic acid; Gal, Galactose.

Designation	Structure	Molar %	No. per α-chain*
N1	$GalNAc_{OL}$	18.9	1.0
N2	$Gal\beta1 \overset{}{\underset{B}{\dashv}} 3GalNAc_{OL}$	17.1	1.0
A1	$NeuNAc\alpha2 \overset{}{\underset{A}{\dashv}} 3Gal\beta1 \overset{}{\underset{B}{\dashv}} 3GalNAc_{OL}$	54.5	2.5
A2	$NeuNAc\alpha2 \overset{}{\underset{A}{\dashv}} 3Gal\beta1 \overset{}{\underset{B}{\dashv}} 3GalNAc_{OL}$ (NeuNAcα2 also $\dashv$ 6 GalNAc$_{OL}$)	9.2	0.5
	Total	100.0	5.0

*Calculated assuming five *O*-linked glycans per α-chain [2], and that all alkali-released oligosaccharides were associated with the heavy chain of IgA1. Dashed lines indicate the positions of action of the exoglycosidases used in the structural analysis. A, *A. ureafaciens* neuraminidase; B, bovine epididymal β-galactosidase.