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Review

Immobilized lectin columns: useful tools for the fractionation and structural analysis of oligosaccharides

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ABSTRACT

Elucidation of the binding specificity of a concanavalin A–Sepharose column led to the possibility of the affinity chromatography of oligosaccharides and glycopeptides with the use of immobilized lectin columns. Subsequent addition of immobilized erythroagglutinating phytohaemagglutinin, *Aleuria aurantia* lectin, *Datura stramonium* agglutinin, *Ricinus communis* agglutinin and *Allomyrina dichotoma* agglutinin to the range of well characterized lectin columns has afforded a way to fractionate a mixture of N-linked oligosaccharides even to a single component.

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1. INTRODUCTION

Concanavalin A (Con A) specifically binds the structure shown in Fig. 1. Accordingly, Glcα1→, Manα→ and GlcNAcα1→ residues, which occur at the non-reducing termini of sugar chains, and →2Glcα1→ and →2Manα1→ residues, which occur within sugar chains, can bind to this lectin.

We previously investigated the behaviour of various glycopeptides and oligosaccharides in a column containing Con A–Sepharose, and reached the important conclusion that the presence of two binding residues is required for a sugar chain to be retained

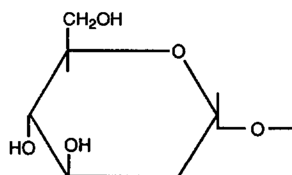

Fig. 1. The structure recognized by Con A.

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in the column [1]. Based on this finding, affinity chromatography with use of a Con A–Sepharose column was developed as an effective method to fractionate and characterize N-linked sugar chains of glycoproteins [2].

Among the N-linked sugar chains listed in Table 1, all of the high-mannose type, monoantennary complex type (1–5) and biantennary complex type (9, 10, 13, 14, 19–23, 32–34, 52–57 and 60) sugar chains and part of the hybrid-type (69–75) sugar chains are retained in the column, as they contain at least two binding residues. The mono- and biantennary oligosaccharides were eluted with 5 m*M* α-methylglucoside solution and high-mannose type oligosaccharides with 200 m*M* α-methylmannoside solution. This method has been widely applied for the study of N-linked sugar chains of glycoproteins, and subsequently further evidence has been found. The presence and absence fo the α-fucosyl residue linked at the C-6 position of the proximal N-acetylglucosamine residue does not affect the binding of an oligosaccharide to the column. As the presence of the β-N-acetylglucosamine residue linked at the C-4 position of the β-mannosyl residue of the trimannosyl core (bisecting GlcNAc) decreases considerably the affinity of an oligosaccharide to the

TABLE 1

NUMBERS ASSIGNED TO OLIGOSACCHARIDES AND THEIR STRUCTURES

R_1 = GlcNAcβ1→4GlcNAc$_{OT}$; R_2 = GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$; R_3 = GlcNAc$_{OT}$

Type	Structure	Number
High-mannose type	(Manα1→2)$_{0\sim1}$ Manα1↘6 Manα1↘6 (Manα1→2)$_{0\sim1}$ Manα1↗3 Manβ1→4R_1 (Manα1→2)$_{0\sim2}$ Manα1↗3	
Complex type	Manα1↘6 Manβ1→4 { R_1 / R_2 } GlcNAcβ1→2Manα1↗3	1 2
	Manα1↘6 Manβ1→4 { R_1 / R_2 } Galβ1→4GlcNAcβ1→2Manα1↗3	3 4
	Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4R_2 Manα1↗3	5
	Galβ1→4GlcNAcβ1→2Manα1↘6 GlcNAcβ1→4Manβ1→4R_2 Manα1↗3	6
	Manα1↘6 GlcNAcβ1→4Manβ1→4 { R_1 / R_2 } Galβ1→4GlcNAcβ1→2Manα1↗3	7 8
	GlcNAcβ1→2Manα1↘6 Manβ1→4 { R_1 / R_2 } GlcNAcβ1→2Manα1↗3	9 10

TABLE I (*continued*)

Type	Structure	Number
	GlcNAcβ1→2Manα1↘6 GlcNAcβ1→4Manβ1→4 { R_1 / R_2 GlcNAcβ1→2Manα1↗3	11 (R_1) 12 (R_2)
	GlcNAcβ1→2Manα1↘6 Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1→2Manα1↗3	13 (R_1) 14 (R_2)
	GlcNAcβ1→2Manα1↘6 GlcNAcβ1→4Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1→2Manα1↗3	15 (R_1) 16 (R_2)
	Galβ1→4GlcNAcβ1→2Manα1↘6 GlcNAcβ1→4Manβ1→4 { R_1 / R_2 GlcNAcβ1→2Manα1↗3	17 (R_1) 18 (R_2)
	Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4 { R_1 / R_2 GlcNAcβ1→2Manα1↗3	19 (R_1) 20 (R_2)
	Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4 { R_1 / R_2 / R_3 Galβ1→4GlcNAcβ1→2Manα1↗3	21 (R_1) 22 (R_2) 23 (R_3)
	Galβ1→4GlcNAcβ1→2Manα1↘6 GlcNAcβ1→4Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1→2Manα1↗3	24 (R_1) 25 (R_2)
	Fucα1 ↓ 3 Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1→2Manα1↗3	26 (R_1) 27 (R_2)
	Fucα1 ↓ 3 Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1→2Manα1↗3 3 ↑ Fucα1	28 (R_1) 29 (R_2)
	Fucα1 ↓ 3 Galβ1→4GlcNAcβ1→2Manα1↘6 GlcNAcβ1→4Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1→2Manα1↗3 3 ↑ Fucα1	30 (R_1) 31 (R_2)

(*Continued on p. 114*)

TABLE I (*continued*)

Type	Structure	Number
	Galβ1→3GlcNAcβ1→2Manα1↘6 Manβ1→4R_1 Galβ1→3GlcNAcβ1→2Manα1↗3	32
	Fucα1→2Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4R_2 Fucα1→2Galβ1→4GlcNAcβ1→2Manα1↗3	33
	Galα1→3Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4R_1 Galα1→3Galβ1→4GlcNAcβ1→2Manα1↗3	34
	Galβ1→4GlcNAcβ1→2Manα1↘6 Galβ1→4GlcNAcβ1↘4 Manα1↗3 Manβ1→4 { R_1 / R_2 / R_3 Galβ1→4GlcNAcβ1↗2	35 36 37
	Galβ1→4GlcNAcβ1→2Manα1↘6 GlcNAcβ1→4Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1↘4 Manα1↗3 Galβ1→4GlcNAcβ1↗2	38 39
	Galβ1→4GlcNAcβ1↘6 Manα1↘6 Galβ1→4GlcNAcβ1↗2 Manβ1→4 { R_1 / R_2 / R_3 Galβ1→4GlcNAcβ1→2Manα1↗3	40 41 42
	Galβ1→4GlcNAcβ1↘6 Manα1↘6 Galβ1→4GlcNAcβ1↗2 GlcNAcβ1→4Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1→2Manα1↗3	43 44
	Galβ1→4GlcNAcβ1↘6 Manα1↘6 Galβ1→4GlcNAcβ1↗2 Manβ1→4 { R_1 / R_2 / R_3 Galβ1→4GlcNAcβ1↘4 Manα1↗3 Galβ1→4GlcNAcβ1↗2	45 46 47
	Galβ1→4GlcNAcβ1↘6 Manα1↘6 Galβ1→4GlcNAcβ1↗2 GlcNAcβ1→4Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1↘4 Manα1↗3 Galβ1→4GlcNAcβ1↗2	48 49
	Galβ1→4GlcNAcβ1↘6 Manα1↘6 Galβ1→4GlcNAcβ1↗2 Manβ1→4R_1 Galβ1→4GlcNAcβ1↘4 Fucα1↗3 Manα1↗3 Galβ1→4GlcNAcβ1↗2	50

TABLE I (*continued*)

Type	Structure	Number
	Galβ1→4GlcNAcβ1↘6 Fucα1↘3 Manα1↘ Galβ1→4GlcNAcβ1↗2 6 Manβ1→4R_1 Galβ1→4GlcNAcβ1↘4 3 Fucα1↗3 Manα1↗ Galβ1→4GlcNAcβ1↗2	51
	Galβ1→4GlcNAcβ1→3 {Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4 {R_1 / R_3 Galβ1→4GlcNAcβ1→2Manα1↗3}	52 53
	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4 {R_1 / R_3 Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→2Manα1↗3	54 55
	Neu5Acα2→3Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4R_1 Neu5Acα2→3Galβ1→4GlcNAcβ1→2Manα1↗3	56
	Neu5Acα2→6 {Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4R_1 Galβ1→4GlcNAcβ1→2Manα1↗3}	57
	Neu5Acα2→6Galβ1→4GlcNAcβ1→2Manα1↘6 GlcNAcβ1→4Manβ1→4R_2 Galβ1→4GlcNAcβ1→2Manα1↗3	58
	Galβ1→4GlcNAcβ1→2Manα1↘6 GlcNAcβ1→4Manβ1→4R_2 Neu5Acα2→6Galβ1→4GlcNAcβ1→2Manα1↗3	59
	Neu5Acα2→6Galβ1→4GlcNAcβ1→2Manα1↘6 Manβ1→4R_1 Neu5Acα2→6Galβ1→4GlcNAcβ1→2Manα1↗3	60
	Neu5Acα2→6Galβ1→4GlcNAcβ1→2Manα1↘6 GlcNAcβ1→4Manβ1→4R_2 Neu5Acα2→6Galβ1→4GlcNAcβ1→2Manα1↗3	61
	Galβ1→4GlcNAcβ1→2Manα1↘6 Neu5Acα2→3Galβ1→4GlcNAcβ1↘4 Manβ1→4R_1 Manα1↗3 Galβ1→4GlcNAcβ1↗2	62
	Galβ1→4GlcNAcβ1→2Manα1↘6 Galβ1→4GlcNAcβ1↘4 Manβ1→4R_1 Manα1↗3 Neu5Acα2→6Galβ1→4GlcNAcβ1↗2	63
	Neu5Acα2→6Galβ1→4GlcNAcβ1→2Manα1↘6 Galβ1→4GlcNAcβ1↘4 Manβ1→4R_1 Manα1↗3 Galβ1→4GlcNAcβ1↗2	64

(*Continued on p. 116*)

TABLE I (*continued*)

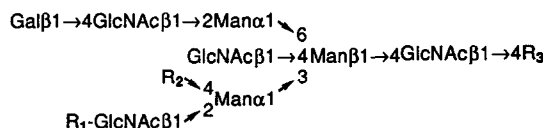
Type	Structure	Number
	Galβ1→4GlcNAcβ1↘6 Manα1↘6 Neu5Acα2→6Galβ1→4GlcNAcβ1↗2 Manβ1→4R_1 Galβ1→4GlcNAcβ1↘4 Manα1↗3 Neu5Acα2→6Galβ1→4GlcNAcβ1↗2	65
	Neu5Acα2→6Galβ1→4GlcNAcβ1↘6 Manα1↘6 Galβ1→4GlcNAcβ1↗2 Manβ1→4R_1 Neu5Acα2→6Galβ1→4GlcNAcβ1↘4 Manα1↗3 Galβ1→4GlcNAcβ1↗2	66
	Neu5Acα2→6Galβ1→4GlcNAcβ1↘6 Manα1↘6 Neu5Acα2→6Galβ1→4GlcNAcβ1↗2 Manβ1→4R_1 Galβ1→4GlcNAcβ1↘4 Manα1↗3 Galβ1→4GlcNAcβ1↗2	67
	Galβ1→4GlcNAcβ1↘6 Manα1↘6 Galβ1→4GlcNAcβ1↗2 Manβ1→4R_1 Neu5Acα2→6Galβ1→4GlcNAcβ1↘4 Manα1↗3 Neu5Acα2→6Galβ1→4GlcNAcβ1↗2	68
	Manα1→3Manα1↘6 Manβ1→4R_1 GlcNAcβ1→2Manα1↗3	69
	Manα1↘6 Manα1↘6 Manα1↗3 Manβ1→4R_1 GlcNAcβ1→2Manα1↗3	70
	Manα1→3Manα1↘6 Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1→2Manα1↗3	71 72
	Manα1↘6 Manα1↘6 Manα1↗3 Manβ1→4 { R_1 / R_2 Galβ1→4GlcNAcβ1→2Manα1↗3	73 74
	Manα1↘6 Manα1↘6 Manα1↗3 GlcNAcβ1→4Manβ1→4R_1 GlcNAcβ1↘4 Manα1↗3 GlcNAcβ1↗2	75
	Manα1→3Manα1↘6 GlcNAcβ1→4Manβ1→4R_1 Galβ1→4GlcNAcβ1↘4 Manα1↗3 GlcNAcβ1↗2	76

column, oligosaccharides 24 and 25 are not retained in the column but are eluted with buffer as retarded fractions. Sialylation and fucosylation of the galactose residues of the outer chain moieties does not affect seriously the affinity of a complex-type sugar chain. In contrast, fucosylation of the N-acetylglucosamine residues in the outer chain (oligosaccharides 28 and 29) decreases considerably the affinity of an oligosaccharide to the column.

The introduction of a Con A-Sepharose column in the field of glucoconjugate research has attracted the attention of many biochemists, and the behaviours of N-linked oligosaccharides in various immobilized lectin columns have been reported. In this paper we review our contributions to this important field.

2. ERYTHROAGGLUTINATING PHYTOHAEMAGGLUTININ (E-PHA)

It has long been known that the extract of red kidney bean (*Phaseolus vulgaris*) agglutinates erythrocytes and induces blastogenesis of lymphocytes. Leavitt *et al.* [3] found that the extract contains five tetrameric isolectins, L_4, L_3E, L_2E_2, LE_3 and E_4. The L and the E subunits bind specifically to lymphocytes and erythrocytes, respectively. Therefore, L_4 (L-PHA) binds with high affinity to lymphocytes and induces blastogenesis in these cells. In contrast, E_4 (E-PHA) specifically agglutinates erythrocytes, but does not show any blastogenic activity. Irimura *et al.* [4] reported that the oligosaccharide 25 in Table 1 is retarded in an E-PHA–agarose column, whereas it passes through the column after β-galactosidase digestion. Cummings and Kornfeld [5] also reported that the biantennary complex-type sugar chains with bisecting GlcNAc are retarded in the column. In order to elucidate the precise binding specificity of the column, we examined the behaviours of many oligosaccharides with bisecting GlcNAc in an E-PHA–agarose column at room temperature. Based on the data that oligosaccharides 18, 25, 39 and 59 are retarded in the column but oligosaccharides 6, 16, 31, 44, 58, 61, 75 and 76 are not, we concluded that the minimum structural unit required for the binding to an E-PHA column is the following octasaccharide:



where R_1 and R_2 represent either hydrogen or sugars and R_3 either ($\pm$Fucα1$\rightarrow$6)GlcNAc$\rightarrow$Asn or ($\pm$Fucα1$\rightarrow$6)GlcNAc$_{OH}$ [6]. In accord with the empirical rule, it was confirmed later that oligosaccharides 17, 24 and 38 are retarded in the column and oligosaccharides 7, 8, 11, 12, 15, 30 and 43 pass through the column.

Interestingly, the behaviour of oligosaccharides in an E-PHA-agarose column at 2°C was different to that at room temperature (20°C) [7]. Oligosaccharides 19–23, which pass through an E-PHA column at room temperature (Fig. 2A, dashed line), are retarded in the column at 2°C (Fig. 2A, solid line). An interesting piece of evidence is that oligosaccharide 5 is also retarded in the column (Fig. 2C, solid line). The affinity of this oligosaccharide is not

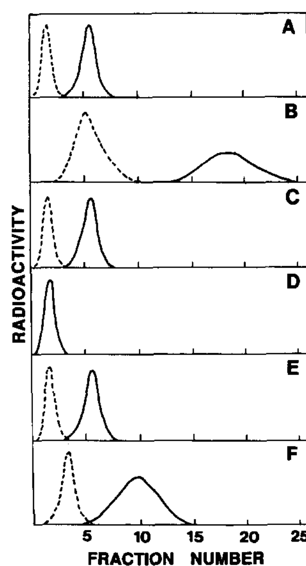


Fig. 2. Affinity chromatography of radioactive oligosaccharides on an E-PHA–agarose column. Dashed lines represent the elution patterns of oligosaccharides at 20°C and solid lines those at 2°C. A, Oligosaccharides 9–23; B, oligosaccharide 25; C, oligosaccharide 5; D, oligosaccharides 32, 33, 40 and 41; E, oligosaccharides 35 and 36; F, oligosaccharide 56.

```
                                                -79
                                     CCCTGAGCTTAAGCCCGCA
AATTTCGAGACCTTGATACCCTCACATCCTACCGCTAGCTACCACACCACCACCCAAGCA

                                                     50
ATGCCTACCGAATTCCTCTACACCTCGAAAATTGCAGCCATCTCTTGGGCTGCCACCGGC
MetProThrGluPheLeuTyrThrSerLysIleAlaAlaIleSerTrpAlaAlaThrGly
 -1  1
                                   100
GGCCGCCAGCAACGCGTCTACTTCCAAGACCTTAATGGCAAGATCCGCGAGGCTCAGCGC
GlyArgGlnGlnArgValTyrPheGlnAspLeuAsnGlyLysIleArgGluAlaGlnArg

                        150
GGGGGAGACAATCCATGGACCGGCGGGTCGAGCCAGAATGTAATCGGCGAAGCAAAGCTT
GlyGlyAspAsnProTrpThrGlyGlySerSerGlnAsnValIleGlyGluAlaLysLeu

              200
TTTTCGCCACTGGCTGCTGTCACGTGGAAAAGTGCTCAGGGCATACAGATCCGTGTTTAC
PheSerProLeuAlaAlaValThrTrpLysSerAlaGlnGlyIleGlnIleArgValTyr

     250                                                300
TGCGTCAATAAGGATAACATCCTCTCCGAATTTGTGTATGACGGTTCGAAGTGGATCACC
CysValAsnLysAspAsnIleLeuSerGluPheValTyrAspGlySerLysTrpIleThr

                                                 350
GGACAGCTGGGCAGTGTCGGCGTCAAGGTGGGCTCCAATTCGAAGCTTGCTGCGCTTCAG
GlyGlnLeuGlySerValGlyValLysValGlySerAsnSerLysLeuAlaAlaLeuGln
100
                                 400
TGGGGCGGATCTGAGAGCGCCCCCCCAAACATCCGAGTTTACTACCAGAAGAGCAACGGT
TrpGlyGlySerGluSerAlaProProAsnIleArgValTyrTyrGlnLysSerAsnGly

                       450
AGTGGGAGCTCAATCCACGAGTATGTCTGGTCGGGCAAATGGACGGCTGGCGCAAGCTTT
SerGlySerSerIleHisGluTyrValTrpSerGlyLysTrpThrAlaGlyAlaSerPhe

                   500
GGGTCAACGGTGCCAGGAACGGGTATCGGAGCCACCGCCATCGGGCCAGGTCGCCTGAGG
GlySerThrValProGlyThrGlyIleGlyAlaThrAlaIleGlyProGlyArgLeuArg

     550                                                600
ATCTACTACCAGGCTACTGACAACAAGATCCGTGAGCACTGTTGGGACTCCAACAGTTGG
IleTyrTyrGlnAlaThrAspAsnLysIleArgGluHisCysTrpAspSerAsnSerTrp

                                                 650
TACGTGGGGGGGTTCTCGGCCAGCGCTTCCGCCGGCGTCTCCATCGCGGCGATTTCTTGG
TyrValGlyGlyPheSerAlaSerAlaSerAlaGlyValSerIleAlaAlaIleSerTrp
200

                  700
GGCAGTACACCCAACATCCGGGTCTACTGGCAGAAAGGTAGGGAGGAATTGTACGAGGCT
GlySerThrProAsnIleArgValTyrTrpGlnLysGlyArgGluGluLeuTyrGluAla

                                  750
GCCTATGGCGGTTCATGGAACACTCCTGGTCAGATCAAGGACGCATCCAGGCCTACGCCC
AlaTyrGlyGlySerTrpAsnThrProGlyGlnIleLysAspAlaSerArgProThrPro

                  800
TCGTTGCCAGACACCTTTATTGCTGCGAACTCCTCGGGGAACATCGACATCTCTGTGTTC
SerLeuProAspThrPheIleAlaAlaAsnSerSerGlyAsnIleAspIleSerValPhe

     850                                                900
TTCCAAGCTAGCGGCGTCTCCTTGCAGCAGTGGCAATGGATCTCCGGCAAGGGCTGGTCC
PheGlnAlaSerGlyValSerLeuGlnGlnTrpGlnTrpIleSerGlyLysGlyTrpSer

                                 939
ATCGGCGCGGTTGTTCCCACTGGCACTCCCGCGGGATGGTAAATGGCGACCAGATACGGC
IleGlyAlaValValProThrGlyThrProAlaGlyTrp
300                                  312

CACATGGAGTGCCCACCTCGGTTCGTAGGGAAAGGCTCAGGCACGTTTCTAGGCTTTTTT

ATTTCTGCTTTCTCTTCTCTCCTTACCATGCGTACGCGGAAAAAGATGGGCGAAAACTCT

CACTATTTATCTGGTTTATCTGTTGTGTGTACGGTGATTTCCTCGGTTGGAGATCACAGG

GTTTCTTTTCCGTTTTGGGGACATGGTTTATTTTACGACTTTCTTTACCCTGGCATACGG

AGTGCTGGATGTTATGGATGCGAATGTGGCTTCTTTGCAGAATATCAGGATATATCTCGG

TGGAAAAAAAAAAAAAAAAAAAAAAAAAAA
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Fig. 3. Nucleotide sequence of AAL cDNA and its deduced amino acid sequence. The amino acid residues which were confirmed by amino acid sequencing of the AAL purified from the mushroom are underlined [11].

lost even if the Manα1→3 residue is removed by α-mannosidase digestion. These results indicate that the requirement of the GlcNAcβ1→2Manα1→3 group, as revealed in the chromatography at room temperature, is lost at 2°C. This may be one of the reasons why the requirement of bisecting GlcNAc is also lost at 2°C. In chromatography at room temperature, bisecting GlcNAc might be required to arrange the two outer chain moieties properly to bind to E-PHA.

In any event, more effective fractionation of N-linked oligosaccharides can be obtained by performing affinity chromatography on an E-PHA–agarose column both at room temperature and at 2°C. That oligosaccharides 35 and 36 are retarded (Fig. 2E, solid line) and oligosaccharides 32, 33, 40 and 41 pass through the column at 2°C (Fig. 2D, solid line) can be explained by the empirical rule described above. Interesting evidence is that oligosaccharide 34 is retarded and oligosaccharide 56 leaves the column later than oligosaccharide 21 (Fig. 2F, solid line). These results indicate that substitution at the C-3 position of the galactose residue of the Galβ1→4GlcNAcβ1→2Manα1→6 group by other sugars does not affect the affinity of an oligosaccharide to E-PHA.

3. *ALEURIA AURANTIA* LECTIN (AAL)

AAL was found by Kochibe and Furukawa [8] as a lectin which agglutinates human erythrocytes irrespective of their ABO and Lewis blood types. They purified the lectin by affinity chromatography using blood group H-active glycopeptide coupled to Sepharose 4B.

The carbohydrate-binding specificity of AAL was investigated by analysing the behaviour of a variety of fucose-containing oligosaccharides on an AAL-Sepharose column [9]. Studies of complex-type oligosaccharides released from glycoproteins by hydrazinolysis revealed that the presence of the α-fucosyl residue linked at the C-6 position of the proximal N-acetylglucosamine moiety is essential for binding to the lectin column. Binding was not affected either by the structure of the outer chain moieties or by the presence of bisecting GlcNAc residue. The only exception is that the complex-type oligosaccharides with two X or H determinants (oligosaccharides 28–31, 33 and 51) in their outer chain moieties bind to the column and are eluted with buffer containing 0.5 m*M* L-fucose. This is because X and H determinants weakly interact with AAL, as evidenced by the slight retardation of oligosaccharides 26, 27 and 50 in the column. With this binding specificity, an AAL–Sepharose column can be used as an effective tool to separate the complex-type oligosaccharides with a fucosylated trimannosyl core from those with a non-fucosylated trimannosyl core.

AAL is a simple protein composed of two identical subunits. A full-length cDNA has recently been cloned [10]. This clone contained 1370 nucleotides and an open reading frame of 939 nucleotides encoding 313 amino acids (Fig. 3). The amino-terminal sequence of the lectin isolated from the mushroom coincided with the deduced amino acid sequence starting from proline at the second residue, indicating that the mature AAL consists of 312 amino acids. The deduced amino acid sequence shows that AAL includes six inernal homologous regions. The recombinant AAL produced by *E. coli* carrying the AAL expression plasmid showed the same biochemical characteristics and sugar-binding specificity as natural AAL [11].

4. *DATURA STRAMONIUM* AGGLUTININ (DSA)

Crowley *et al.* [12] reported that Galβ1→4GlcNAcβ1→6(Galβ1→4GlcNAcβ→2)Man interacts much more strongly with DSA than the isomeric Galβ1→4GlcNAcβ1→4(Galβ1→4GlcNAcβ1→2)Man. Because this binding specificity was considered to be useful for the fractionation of highly branched complex-type N-linked sugar chains, we investigated the behaviour of a variety of complex-type oligosaccharides on a DSA–Sepharose column [13]. It was confirmed that oligosaccharides were separated into three groups: a pass-through fraction, a retarded fraction and a bound fraction wich is eluted with buffer containing a mixture of N-acetylglucosamine oligomers (fractions I, II and III, respectively, in Fig. 4). All oligosaccharides which contain the Galβ1→4GlcNAcβ1→4(Galβ1→4GlcNAcβ1→2)Man group (oligosaccharides 35–39) are retarded in the column so long as this pentasaccharide group is not substituted or substituted only at the C-3 position of the galactose residues by other sugars (oligosaccharide 62).

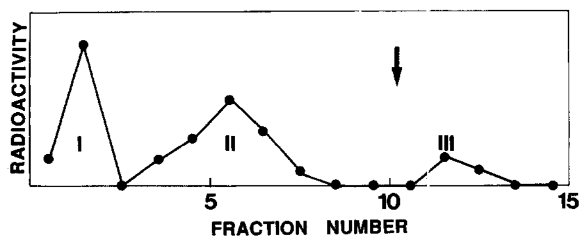

Fig. 4. Three fractions of complex-type oligosaccharides obtained by DSA-Sepharose column chromatography. The arrow indicates the position where the elution buffer was switched to that containing 1% of a mixture of diacetylchitobiose, triacetylchitotriose and tetraacetylchitotetraose.

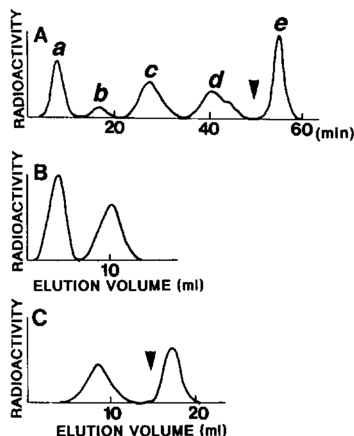

Fig. 5. Serial lectin affinity chromatography of the mixture of radioactive fucosylated oligosaccharides obtained from a desialylated IgG sample by hydrazinolysis. A, Fractionation pattern obtained by RCA I–agarose column chromatography; B, elution patterns of peaks d and e in (A) from an E-PHA–agarose column; C, elution pattern of peak a in (A) from a Con A–Sepharose column. The arrows in A and C indicate the positions where the elution buffers were switched to those containing 10 m*M* lactose and 200 m*M* α-methylglucoside, respectively.

Oligosaccharides which contain the unsubstituted Galβ1→4GlcNAcβ1→6(Galβ1→4GlcNAcβ1→2)Man group (oligosaccharides 40–50) or the Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAc group (oligosaccharides 52–55) are all recovered in the bound fraction. Substitution at the C-3 position of the galactose residues of the pentasaccharide and the tetrasaccharide groups does not affect the binding character of the oligosaccharides, but substitution of any other portion of these groups totally deprives the affinity with DSA from the oligosaccharides. Accordingly, oligosaccharide 63 can be separated from its isomeric oligosaccharide 62 or 64. Among the four isomeric disialylated tetraantennary oligosaccharides listed in Table 1, oligosaccharides 65 and 66 fall into fraction I and oligosaccharides 67 and 68 into fractions II and III, respectively.

5. *RICINUS COMMUNIS* AGGLUTININ (RCA I)

A column containing immobilized RCA I retains oligosaccharides with non-reducing terminal β-galactose residues. The affinity of oligosaccharide to the column is enhanced by increasing the number of non-substituted β-galactosyl residues. Accordingly, oligosaccharides 9, 13 and 21 can be readily fractionated by passage through an RCA I–agarose column. An interesting piece of evidence is that monogalactosylated biantennary oligosaccharides 16, 20 and 14 or 18 can be separated as shown in Fig. 5A [14]. Because the fucose residues of these oligosaccharides are not recognized by the column, oligosaccharides 15, 19 and 13 or 17 behave in exactly the same manner (not shown).

6. *ALLOMYRINA DICHOTOMA* AGGLUTININ (ALLO A-II)

The haemolymph of the beetle *Allomyrina dichotoma* contains two lectins, Allo A-I and Allo A-II [15]. Both lectins agglutinate human erythrocytes. Investigation of the behaviour of various oligosaccharides on an immobilized Allo A-II column as described below revealed that the column is very useful for the analysis of sialylated oligosaccharides [16].

In principle, Allo A-II weakly interacts with an N-acetyllactosamine group. Therefore, the elution volume of non-sialylated complex-type oligosaccharides with the Galβ1→4GlcNAc outer chain increases in proportion to the number of the disaccharide group in a molecule (oligosaccharides 3 and 4 < oligosaccharides 21 and 22 < oligosaccharides 35, 36, 40 and 41 < oligosaccharides 45 and 46). The importance of the Galβ1→4GlcNAc group is also indicated from the evidence that oligosaccharides 9 and 10 pass through the column without interaction. Oligosaccharides 28, 29, 33, 34 and 56 pass through the column, indicating that substitution of the Galβ1→4GlcNAc group by other sugars

abolishes its interaction with Allo A-II. An interesting piece of evidence is that oligosaccharides 57, 59, 60, 61, 63, 65, 66, 67 and 68 bind strongly to the column. Accordingly, the column can be used as an effective tool to discriminate acidic oligosaccharides with a Neu5Acα2→3Galβ1→4GlcNAc group from those with a Neu5Acα2→6Galβ1→4GlcNAc group. Oligosaccharide 60, which strongly binds to the column, cannot be eluted with buffer containing 0.2 *M* N-acetylneuraminic acid. This indicated that the sialic acid linked at the C-6 position of the galactose residue of the Galβ1→4GlcNAc group plays only an auxiliary role, either by causing the binding disaccharide group to assume a conformation that fits to the binding site of Allo A-II, or by interacting with some charged amino acids of the lectin to help orient the binding of the disaccharide group.

7. FRACTIONATION OF RADIOACTIVE OLIGOSACCHARIDES RELEASED FROM HUMAN IMMUNOGLOBULIN G BY HYDRAZINOLYSIS

As an example to show the usefulness of serial lectin column chromatography for the fractionation of the N-linked sugar chains of glycoproteins, a study was made of the sugar chains of human immunoglobulin G (IgG).

IgG is a glycoprotein composed of two subunits, heavy (H) and light (L), with a stoichiometry of H_2L_2. Each H subunit contains an N-linked sugar chain at Asn 297. Study of the whole sugar chains of the IgGs purified from sera of healthy individuals revealed that several unique characteristics are included in these sugar chains. The largest sugar chain of human IgG is oligosaccharide 61 in Table 1. One of the most characteristic features of the sugar chain of IgG is its extremely high structural multiplicity. This multiplicity is produced by the presence or absence of sialic acid, galactose, fucose and bisecting GlcNAc residues of the biantennary sugar chains. Therefore, even after desialylation, a mixture of sixteen different oligosaccharides (oligosaccharides 9–22, 24 and 25 in Table 1) are obtained by hydrazinolysis of serum IgG samples. These oligosaccharides could be completely separated by serial lectin column chromatography [14]. First, all fucosylated oligosaccharides were separated from non-fucosylated oligosaccharides by passage through an AAL–Sepharose column. In the following procedure, both fractions gave exactly the same fractionation pattern. Accordingly, the data for fucosylated oligosaccharides (a mixture of oligosaccharides 10, 12, 14, 16, 18, 20, 22 and 25 in Table 1) are given in Fig. 5. When the oligosaccharide mixture was subjected to affinity chromatography with use of an RCA I column, it was separated into five peaks (Fig. 5A). It was found that peaks b and c contain oligosaccharides 16 and 20, respectively. By E-PHA–agarose column chromatography, both peaks d and e were separated into pass-through and retarded components (Fig. 5B). The unbound and retarded fractions of peak d were oligosaccharides 14 and 18, respectively. The unbound and retarded fractions of peak e were oligosaccharides 22 and 25, respectively. Peak a in Fig. 5A was also separated into retarded and bound components by Con A–Sepharose column chromatography (Fig. 5C). The retarded and the bound fraction were found to contain oligosaccharides 12 and 10, respectively. Therefore, the sixteen neutral oligosaccharides can be completely separated by serial affinity chromatography with the use of immobilized AAL, RCA I, E-PHA and Con A columns. As the method was simple and precise data on the percentage molar ratio of the sixteen oligosaccharides were obtained by using several thousand counts per minute of total radioactive oligosaccharides released from 1 nmol of IgG, it was effectively used to analyse the sugar patterns of IgG samples obtained from healthy individuals and patients with rheumatoid arthritis [17].

8. CONCLUDING REMARKS

The combination of serial lectin column chromatography with hydrazinolysis affords a simple and very sensitive method for fractionating various N-linked oligosaccharides found in glycoproteins. Based on the strict binding specificities of lectins, the method can also be used for the structural study of an oligosaccharide and a glycopeptide. The usefulness of a DSA–Sepharose column for the discrimination of hCGs in urine samples obtained from patients with various trophoblastic diseases was also verified by our study [18]. The addition of further useful lectin columns to those currently available will surely be of great value for the analysis of the sugar chains of glycoproteins.

9. ACKNOWLEDGEMENT

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