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Oligosaccharide structure determination of glycoconjugates using lectins

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Abstract. Lectins, the divalent or polyvalent (glyco) proteins of non-immune origin of the cells agglutinate cells or other materials, that display more than one saccharide of sufficient complementarity. Lectins considered 'identical' in terms of mono- and disaccharide specificity can be differentiated by their ability to recognise the fine differences in more complex structures. The present review discusses the interaction of lectins with various oligosaccharides and their resultant separations due to structural variations.

Keywords. Oligosaccharide structures; lectins; glycoconjugates.

Lectins, the divalent or multivalent (glyco) proteins of the cells are of non-immune origin. Proteins of this class share a common ability to agglutinate cells or other materials that display more than one saccharide of sufficient complementarity. Such agglutinins are found predominantly in seeds of plants, in particular those of legumes, but are also present in other parts of plants and other living organisms inclusive of human. There are excellent reviews on the isolation, cell agglutination and other functions of lectins (Sharon and Lis, 1972, 1973; Barondes, 1981, 1984). Lectins considered 'identical' in terms of monosaccharide specificity possess the ability to recognise fine differences in more complex structures. In this review we are discussing the interaction of lectins with various glycoproteins, glycopeptides and their separation due to oligosaccharide structures.

The presence of Concanavalin A (ConA) in the seeds of *Canavalia ensiformis* was demonstrated by Sumner and Howell (1936). The homogeneous purification and the utilisation of affinity chromatography as technique was achieved by Agarwal and Goldstein (1967). Since then several lectins had been purified and characterised. The statement 'nothing is known about their role in nature' by Lis and Sharon (1973) has been upgraded in recent times (Barondes, 1981, 1984). The aspect of the function of lectin as analytical tool in affinity chromatography has also emerged mostly from the work of Stuart Kornfeld and his co-workers at Washington University, St. Louis, USA. Oligosaccharide structures mentioned in the text are given in tables 1–3.

ConA is the most widely studied lectin. The structural requirements of mono- and disaccharides had been described (Goldstein and Hayes 1978; Brewer and Brown 1979; Loontiens *et al*., 1983). However, its binding affinity to oligosaccharides seems to depend significantly on the type of linkages and immediate neighbouring saccharides.

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Abbreviations used: ConA, Concanavalin A; galNAc, N-acetyl galactosamine; gal, galactose; NeuAc, N-acetylneuraminic acid; man, mannose, Asn, asparagine; fuc, fucose; glc, glucose; glcNAc, N-acetyl glucosamine; RCAI, *Ricinus communis* agglutinin; RCAII, *R. communis* toxin; WBA, Winged bean agglutinin; WGA, Wheat germ agglutinin; PHA, *Phaseolus vulgaris*; E-PHA, erythrocyte PHA; L-PHA, leucocyte PHA

Table 1. Oligosaccharide structures.

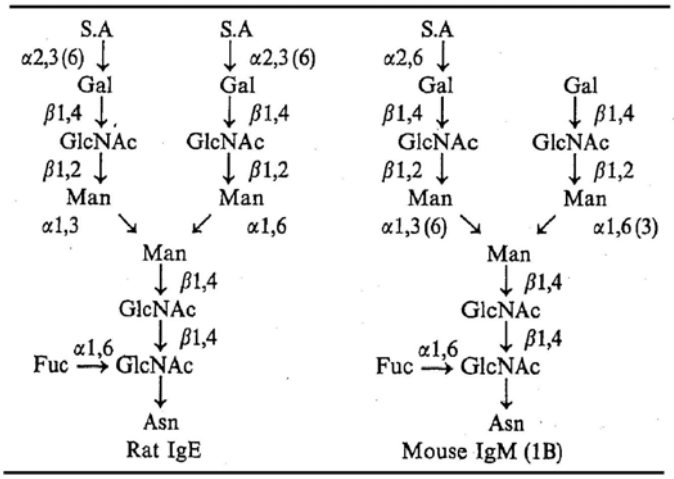
Abbreviation	*Structure*
M-1	α-Man-(1→3)-β-Man-(1→4)-GlcNAc
M-2	α-Man-(1→2)-α-Man-(1→3)-β-Man-(1→4)-GlcNAc
M-3	α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→3)-β-Man-(1→4)-GlcNAc
N-1	α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)-β-Man-(1→4)-GlcNAc
N-2	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)-β-Man-(1→4)-GlcNAc
N-3	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-GlcNA α-Man-(1→6)
N-4	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-GlcNA β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)
N-5	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-GlcNA β-GlcNAc-(1→2)-α-Man-(1→6)
N-6	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-GlcNA α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)
S-5a	β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-GlcNAc β-GlcNAc-(1→2)-α-Man-(1→6)
S-4b	β-GlcNAc-(1→2)-α-Man-(1→3) β-GlcNAc-(1→4)-β-Man-(1→4)-GlcNAc β-GlcNAc-(1→2)-α-Man-(1→6)

The lectins With specificity for monosaccharide, α-mannose and α-glucose are ConA, and the agglutinins from lentil (*Lens culinaris*), *Vicia faba*, and pea (*Pisum sativam*). ConA presents a great affinity for trimannosidic core substituted by two N-acetyl glucosamine (glcNAc) residues (S–5a). The affinity is not decreased by the addition of a *β*-(→4) linked glcNAc linked to *β*-man residue (S–4b). But the affinity is reduced when these glcNAc-residues are substituted by galactose (gal)-(N–4, N–5) or by *β*-gal-α-(2→6) N-acetylneuraminic acid (NeuAc)-residues (N–6). But an α(l→6) mannose (man) residue substituted *β*-man core (N-3) was more inhibitory than the core itself (N–2). The oligosaccharides (M-l) without the trimannosidic core even with a terminal α(l →3)-man was a poor inhibitor. The addition of even one or two *α*(l→2)-man did increase inhibitory capacity. But an oligosaccharide structure containing the trimannosidic core with *α*(l→3) and *α*(l→6)-man was a good inhibitor. The substitution of the core *α*(l→3)-man at C–4 position (OVO-TF) made it a poor inhibitor. Lentil, Vicia faba and Pea lectins showed best inhibition when Asparagine (Asn)-linked first *ß*-glcNAc was substituted with *α*(l→6)-fucose(fuc) residue (hLTF 1 and 2). The removal of *α*-fuc residue made it a poor inhibitor (Debray *et al*., 1981). Similar role of *α*-fuc residue attached to Asn-linked *ß*-glcNAc of the core was observed by Kornfeld *et al*. (1981). They also showed that sialylated rat immunoglobulin E and mouse immunoglobulin M (1B), glycopeptides bind to immobilised pea and lentil lectins and eluted only with 500 mM *α*-methyl mannoside in the eluting buffer. Lentil lectin has a hydrophobic region in or near the saccharide binding site as shown by Allen *et al*. (1976), since 3-O-methyl-and 3-O-benzyl derivative D-glucose(glc) was found to be superior inhibitors to D-glc alone. This idea was supported by Debray *et al*. (1981) on account of the presence of *α*-fuc at Asn-

Table 2. Oligosaccharide structures.

Abbreviation	*Structure*
GP-hSTF	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→)-Asn α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)
GP-hLTF-1	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→)-Asn α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) |1,6 x-Fuc
GP-hLTF-2	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→)-Asn β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) |1,6 x-Fuc |1,3 α-Fuc
GP-ovoTF	β-GlcNAc-(1→4) β-GlcNAc-(1→2)-α-Man-(1→3) β-GlcNAc-(1→4)-β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→)-Asn β-GlcNAc-(1→2)-α-Man-(1→6)

Table 3. Oligosaccharide structures.



linked glcNAc. It has also been observed that removal of NeuAc- and gal-from the biantenarry oligosaccharide chains made it poor inhibitor, but removal of NeuAc alone had no effect (h-STE). Lentil and *Vicia faba* agglutinins were not inhibited by oligosaccharides with terminal man-residues in a terminal non-reducing position (M1–M3). The absence of fuc at Asn-N-acetyl glucosamine (glcNAc) (N–6) residue made it 130-times less inhibitory (hLTFl and 2) to lentil lectin. It also showed greater affinity for oligosaccharides with substitution by NeuAc residue at C–6 position of gal rather than at C–3 (N1, N2 and N4). Lentil, pea and *Vicia faba* agglutinins had an hydrophobic area in or near carbohydrate binding site and this site could cause non-specific interaction between lectins and aromatic residues of glycolproteins or glycopeptides (Debray *et al*., 1981). Kornfeld *et al*. (1981) had stated that the above mentioned lectins had closely related primary structure and subunit assembly, hence it was not unexpected that they showed similarity in saccharide-binding specificity.

Ricinus communis agglutinin (RCAI) and *R. communis* toxin (RCAII) have different molecular weights, 120 kDa and 60 kDa, respectively. RCAI showed greater affinity for saccharide sequences possessing gal-residue (s) substituted by NeuAc residue (s) at C–6 rather than at C–3 position (Nl, N2, N4). The above observation was made by Baenziger and Fiete (1979) and had been confirmed by Debray *et al*. (1981). Adair and Kornfeld (1974) had observed that human immunoglobulin G glycan structure was identical to that of a glycopeptide of human lactotransferrin and was 3-fold more inhibitory than the glycopeptide of human transferrin which lacked α-(l→6) fuc near N-glycosidic linkage. Baenziger and Fiete (1979) observed that RCAII had better affinity towards O-glycosidic linkages with β-gal (l→3)-galNAc sequence. RCAII showed higher affinity than RCAI when similar sequence was present in N-glycosidic linkages.

Both RCA lectin and Winged bean agglutinin (WBA) are inhibited by galNAc and simple β-galactosides, like lactose. However, the strong affinity of β-gal terminating asialofetuin oligosaccharide for RCA lectin is totally absent against WBA (Appukuttan, P. S. and Basu, D., unpublished results). Wheat germ agglutinin (WGA) is a glcNAc specific lectin widely used for cell-surface glycoconjugate mapping and oligosaccharide structural determination. The best inhibitor for WGA is chitotriose (Allen *et al*., 1973; Goldstein *et al*., 1975; Monsigny *et al*., 1978). Human transferrin and

lactotransferrin were found to be better inhibitor (Debray *et al*., 1981). Goldstein *et al*. (1975) had observed that WGA interacts with glyco-Asn -*ß*-man-(l→4)-*ß*-glcNAc (l→4) *ß*-glcNAc (l→)-Asn and *ß*-glcNAc (1→4) *ß*-glcNAc (l→)-Asn with equivalent inhibitory power to chitobiose. WGA was found to interact with NeuAc of glycol-conjugates (Bhavanandan and Katlic, 1973; Peters *et al*., 1979). Monsigney *et al*. (1980) had termed this interaction as a 'charge effect'. It had been observed that glycopeptides containing two external glcNAc residues and their asialoagalacto form with two exposed glcNAc residues were not inhibitory to WGA agglutination. However, Yamamoto *et al*. (1981) observed with ovalbumin glycopeptides that the N,N'-diacetyl chitobiose moiety and *ß*-glcNAc residue linked to *β*-man contributed to the interaction of the glycopeptides with WGA-Sepharose.

Phaseolus vulgaris (PHA) contain two lectins one agglutinated erythrocytes (E-PHA) and the other agglutinated leukocytes (L-PHA). High affinity binding to E-PHA occurs only with biantenary glycopeptides with 2 outer gal-residues and a GlcNAc-residue linked *β* (1→4) to *β*-man of the trimannosidic core. The same species did not bind to L-PHA. Tri- and tetraantenary glycopeptides containing outer gal-residues and an *α*-linked man substituted at positions C–2 and C–6 are specifically required for L-PHA (Cummings and Kornfeld, 1982).

Cummings and Kornfeld (1982) had isolated and demonstrated the structures of high man, hybrid and complex types of glycopeptides from mouse lymphoma cells (BW 5147) by serial fractionation through immobilised ConA, WGA, Pea, E-PHA and L-PHA columns. These glycopeptides were all N-asparaginyl linked. ConA bound eluated with 100 mM *α*-methyl mannoside at 60°C contained high man and hybrid type oligosaccharides. These can be further separated by passing through WGA column into unbound high man and bound hybrid type. ConA unbound and 10 mM *α*-methyl mannoside eluate at 25°C contained various types of complex oligosaccharides. They were resolved to near homogeneity by affinity chromatography on pea, E-PHA and L-PHA columns.

Soluble oligosaccharide sequence preferences among naturally occurring glycoproteins for lectins may not be apparent in their affinity for mono-or disaccharide specificities. Thus both *Artocarpus integrafolia* (Jacalin) and *Griffonia simplicifolia* lectins prefer *α*-linked galactose containing mono-ordisaccharides for agglutination inhibition (Sureshkumar *et al*., 1982; Murphy and Goldstein, 1977, 1979). However the marked affinity of Jacalin for immunoglobulin oligosaccharide sequence is absent in *griffonia* lectin (Roque-Barreira and Campos-Neto, 1985). Notably the suggested structure of immunoglobulin did not contain *α*-gal residues (Baenziger and Kornfeld, 1974).

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