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Structural basis for the affinity of four insolubilized lectins, with a specificity for α-D-mannose, towards various glycopeptides with the N-glycosylamine linkage and related oligosaccharides

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Abstract. Precisions are given on the fine specificity and on the usefulness of immobilized concanavalin A, *Lens culinaris, Vicia faba* and *Pisum sativum* agglutinins for fractionation of glycopeptides with the N-glycosylamine linkage.

While insolubilized concanavalin A represents a very useful tool for the fractionation of both N-acetyllactosaminic and oligomannosidic type glycopeptides or related oligosaccharides,immobilized *Lens culinaris,* as well as *Vicia faba* or *Pisum sativum* agglutinins allow the subfractionation of some N-acetyllactosaminic glycopeptide populations on the basis of the presence of an α-L-fucose residue substituting in C-6 position the N-acetylglucosamine residue involved in the N-glycosylamine bond.

Keywords. Affinity chromatography; specificity of lectins; fractionation of glycopeptides.

Introduction

Lectins are now widely used as tools to visualize the topography of saccharide moieties on cell surfaces (Molday, 1981), or to follow by lectin-affinity immunoelectrophoresis variations in the glycosylation of plasma (Bøg-Hansen *et al.,* 1975) or membrane glycoproteins (Bjerrum and Bøg-Hansen, 1976). Insolubilized on various supports, lectins are also widely used to fractionate soluble or membrane glycoproteins of diverse origins (Lotan and Nicolson, 1979) as well as glycolpeptides obtained from soluble or membrane-bound glycoproteins (Finne *et al.,* 1980).

Among the most used lectins are the so-called α-D-mannose or glucose binding lectins: Concanavalin A (Con A), *Lens culinaris* agglutinin (LCA), *Vicia faba* agglutinin (VFA) and *Pisum sativum* agglutinin (PSA) (Goldstein and Hayes, 1978). However, it is now well known that it is not only a monosaccharide residue in a terminal non reducing position on a glycan that is recognized by a given lectin, but more complex saccharidic sequences (Kornfeld and Ferris, 1975).

Abbreviations used: Con A, Concanavalin A; LCA, *Lens culinaris* agglutinin; VFA, *Vicia faba* agglutinin; PSA, *Pisum sativum* agglutinin; GP-h-STF, glycopeptide isolated from human serotransferrin; GP-h-LTF, from human lactotransferrin; GP-ovoTF, hen ovotransferrin; GP-b-LTF, bovine lacto transferrin; FNR, non retained profile, FR, retained profile; FE, sharp elution profile; FTB, "tightly bound" profile.

In previous studies (Debray *et al*.,1979, 1981), we used oligosaccharides or glycopeptides related to glycoproteins with the N-glycosylamine linkage (Montreuil, 1980, 1982) to precise the specificities of various lectins and in particular of the above α-D-mannose binding lectins, by inhibition of agglutination of human red blood cells. These results were completed by studying the affinity of the insolubilized lectins towards these oligosaccharides or glycopeptides.

Materials and methods

Lectins

All the lectins used in this study, except concanavalin A (Con A) which was purchased from Pharmindustrie, Clichy, France, have been purified by affinity chromatography.

Origin of glycopeptides and oligosaccharides

Oligosaccharides (N-9, N-5, N-6, S-6a) shown in figure 1 were isolated by Dr G. Strecker from the urines of patients with various lysosomal diseases (Strecker and Montreuil, 1979). Glycopeptides isolated from human serotransferrin (GP-h-STF), from human lactotransferrin (GP-h-LTF_1) and from hen ovotransferrin (GP-ovoTF) were a gift from Prof. G. Spik. The glycopeptide of the oligomannosidic type, isolated from bovine lactotransferrin (GP-b-LTF) was a gift from Dr A. Chéron.

All these structures shown in figure 1 are related to glycoproteins with the N-glycosidic linkage and belong to 2 types of glycan structures: the oligomannosidic and the N-acetyllactosaminic type.

In the first family, the common pentasaccharidic core is substituted uniquely by mannose residues to give the oligomannosidic type as found in bovine lactotransferrin (GP-b-LTF of figure 1) or by a variable number of residues of N-acetyllactosamine and of sialic acid and/or fucose to give the N-acetyllactosaminic type (Montreuil, 1980, 1982). In the case of human serotransferrin, we found two of these N-acetyllactosamine sequences substituted by two residues of sialic acid, giving a bi-antennary N-acetyllactosaminic type glycan (GP-h-STF of figure 1).

With one or two other N-acetyllactosaminic sequences, are obtained the tri- or tetra-antennary glycans as those characterized in human α_1-acid glycoprotein (Fournet *et al*.,1978). Very often, on a bi-antennary glycan, a L-fucose residue is branched in C-6 position on the N-acetylglucosamine residue involved in the N-glycosylamine bond. This type of glycan has been found in human lactotransferrin (GP-h-LTF of figure 1) (Spik *et al*.,1982). Sometimes, the core β-mannose residue is substituted by an intersecting β-N-acetylglycosamine residue as in the glycan isolated from hen ovotransferrin (GP-ovoTF of figure 1).

Haemagglutination tests

Agglutination of human red blood cells group O^+ and haemagglutination inhibition by various oligosaccharides or glycopeptides were carried out according to Matsumoto and Osawa (1970). Results were expressed as the minimum concentration of sugar (mM) necessary to completely inhibit agglutination.

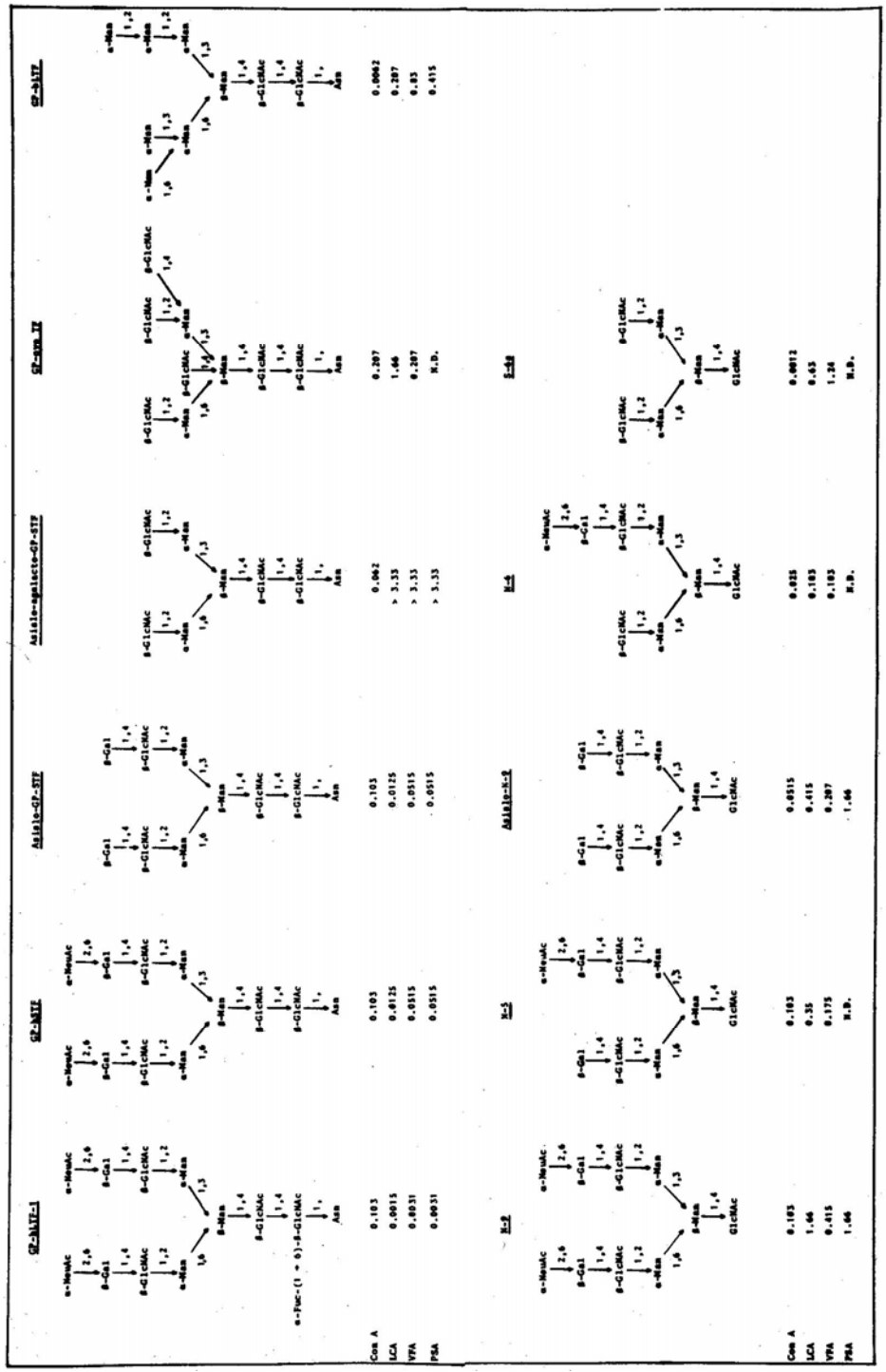

Figure 1. Saccharidic determinants recognized by Con A, LCA, VFA and PSA on glycans from glycoproteins with the N-glycosylamine linkage.

Insolubilization of lectins on Sepharose 4B

Lectins were coupled to Sepharose 4B activated according to the procedure of March *et al.* (1974), at a concentration of 2 mg of lectin per ml of gel.

Affinity chromatography of oligosaccharides or glycopeptides on lectin-Sepharose column

Glycopeptides, N-^{14}C acetylated according to Koide *et al.* (1974), or related oligosaccharides, reduced on the terminal N-acetylglucosamine residue with tritiated sodium borohydride (Takasaki and Kobata, 1974) were applied, after dissolution in phosphate buffered saline pH 7.4 (LCA, VFA, PSA) or in 0.005 M sodium acetate buffer pH 5.2 containing 0.1 M-NaCl and $CaCl_2$, $MgCl_2$ (1 mM each) in the case of insolubilized Con A, to the column of lectin-Sepharose (10×1 cm) equilibrated with the same buffer. Elution was carried out first with buffer and then with buffer containing 0.15 M methyl-α-D-glucoside. Fractions of 2 ml were collected and samples of 100 µl analysed for radioactivity.

Results and discussion

Haemagglutination inhibition results

These results are summarized in figure 1. The best inhibitors found for Con A are asialo-agalacto GP-h-STF and the related oligosaccharide S-6a and Con A presents a great affinity for the trimannosidic core substituted by two N-acetylglucosaminyl residues. The affinity of the lectin decreases when these N-acetylglucosamine residues are substituted by one or two galactose residues as in asialo-GP-STF and asialo N-9 (figure 1) and by one or two sialic acid residues as in GP-h-STF and N-9 of figure 1. It is noteworthy that this affinity, as measured by inhibition of agglutination, is not decreased by the addition of a β-l,4-linked N-acetylglucosamine on the β-linked mannose residue. On the other hand, Con A also presents a great affinity for GP-b-LTF which possesses the complete trimannosidic core with α-1,3 and α-1,6-mannose substituted by four additional α-linked mannose residues (oligomannosidic structure). Substitution of core β-1, 3-mannose at position C-4, as in GP-ovoTF, decreases the affinity of Con A.

However, the best inhibitor found for the three other lectins LCA, VFA and PSA is glycopeptide GP-h-LTF_1 and the α-1,6-linked fucose residue seems to be a major determinant of the binding, for its removal with an α-L-fucosidase from rat kidney gives glycopeptides 10-fold less inhibitory (GP-h-STF). However, if presence of an α-1,6-linked fucose near the N-glycosidic linkage seems to be a necessary condition, its presence is not a sufficient one, and glycopeptides possessing this fucose residue, but where terminal neuraminyllactosamine sequences are lacking are very poor inhibitors (Debray *et al.,* 1981). This suggests the existence of an extended carbohydrate binding site for LCA, VFA and PSA and the methyl group of L-fucose near the N-glycosidic linkage could interact with an hydrophobic area in or near this carbohydrate binding site. This hydrophobic area had been previously suggested for LCA, VFA and PSA by Allen *et al.* (1976) . Such an hydrophobic region, close to the carbohydrate-binding site had also been demonstrated for Con A (Loontiens *et al.,* 1973).

Affinity chromatography of glycopeptides with the N-glycosylamine linkage and related oligosaccharides on insolubilized lectins

The haemagglutination inhibition values given in figure 1 can be related to the true association constants between various oligosaccharides or glycopeptides and a given lectin, and from these inhibition values the affinity of a saccharide for the insolubilized lectin could be predicted.

In fact, for insolubilized Con A, results obtained by this second method, confirms previous data (Ogata *et al.,* 1975; Baenziger and Fiete, 1979). The four elution profiles which can be obtained with insolubilized Con A are shown in figure 2: i) The non-retained profile (FNR) is obtained with tetra- or tri-antennary glycopeptides of the N-acetyllactosaminic type or with bi-antennary structures possessing an additional β-1,4-linked N-acetylglycosamine on the β-linked mannose residue. One can note that this last point is in contrast with the haemagglutination

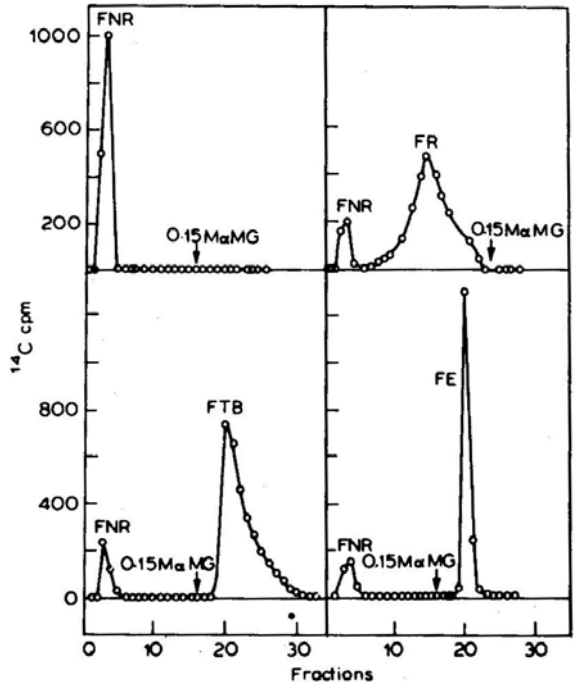

Figure 2. Con A – Sepharose elution profiles.

inhibition results, where this β-1,4 substituted glycan possesses the same inhibitory power than the unsubstituted one (S-6a). This discrepancy is not explained.

ii) The retarded profile (FR) is obtained by elution with the starting buffer and reflects a weak affinity between Con A and the saccharide. This profile is obtained with bi-antennary structures as GP-h-STF (Debray and Montreuil, 1978; Narasimhan *et al.,* 1979). However, with a higher concentration of Con A in the gel, these structures can be weakly retained and then eluted specifically with low concentration of α-methyl-D-glucoside or marinoside (Krusius *et al.,* 1976).

iii) The sharp elution profile (FE) with 0.3 M α-methyl-D-glucoside is obtained with oligomannosidic type glycans as GP-h-LTF (Debray and Montreuil, 1978;

Narasimhan *et al.,* 1979). However, with a higher concentration of Con A in the gel, a broad peak can be obtained (Krusius *et al.,* 1976) and some glycans cannot be eluted with α-methyl-D-mannoside but only with detergents (Ogata *et al.,* 1975).

iv) The "tightly bound" profile (FTB) is obtained with bi-antennary structures such as S-6a or asialo-agalacto-GP-h-STF, where at least one N-acetylglucosamine β (1 → 2) α-mannose sequence is very accessible to the lectin. Same results were described by Narasimhan *et al.* (1979) and Baenziger and Fiete (1979) and this broad, trailing profile expresses a very great affinity between the saccharide and the lectin.

With insolubilized LCA, VFA or PSA, three elution profiles shown in figure 3 can be obtained:

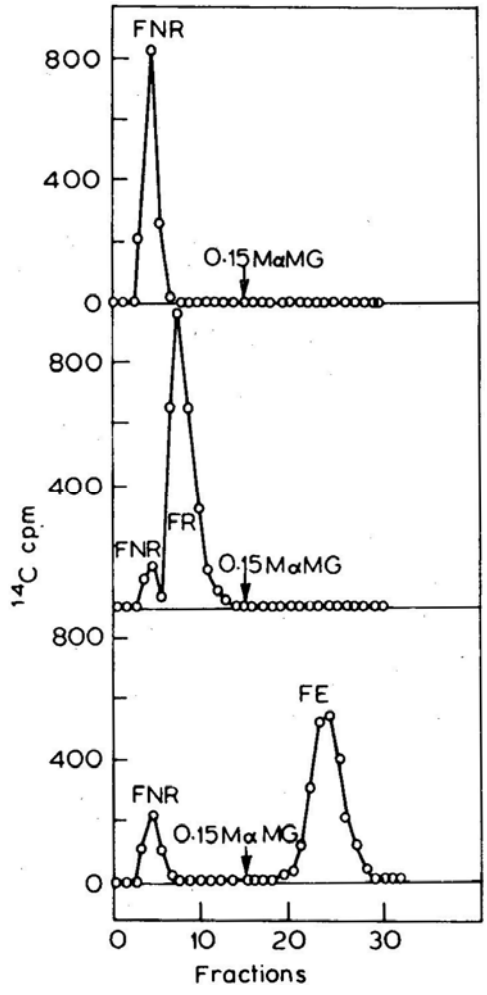

Figure 3. LCA, VFA or PSA – Sepharose elution profiles.

(FNR, Non-retained profile; FR, retained profile; FTB, tightly bound profile; FE, sharp elution profile; α-MG, α-methyl glucoside).

i) The non-retained profile (FNR) is obtained with tetra-, tri- or bi-antennary glycopeptides lacking the α-1,6-linked fucose on the N-acetylglucosamine involved in the N-glycosylamine bond.

ii)The retarded profile is obtained for insolublilized VFA or PSA, with bi-antennary glycopeptides (GP-h-LTF) possessing this α-1,6-linked fucose:

iii)This glycopeptide (GP-h-LTF) is tightly bound (FTB) on insolubilized LCA and is only displaced with 0.15 M α-methyl-N-glucoside.

In the case of insolubilized LCA, after desialylation of GP-h-LTF, the affinity of the lectin for the new structure is not modified. But, after removal of the two galactose residues with a β-galactosidase, the affinity of LCA increases and a broad trailing profile is obtained, reflecting a very great affinity between the saccharide and the lectin. Then, after removal of the N-acetylglucosamine residues with a β-N-acetylglucosaminidase, the affinity of the lectin decreases and finally, if one of the two α-mannose residues is taken off with an α-mannosidase, the remaining structure is no longer retained on the insolubilized LCA.

So, in fact, the saccharidic sequence recognized by LCA and also by VFA and PSA is the pentasaccharidic core substituted by two β-1,2 N-acetylglucosamine residues and possessing an α-L-fucose residue in C-6 position on the N-acetylglucosamine residue involved in the N-glycosylamine bond. However, if the presence of this α-1,6-linked fucose seems to be a necessary condition for the recognition by LCA or PSA and VFA, its presence is not a sufficient one, for the glycan, liberated from glycopeptide GP-h-LTF by hydrazinolysis and which still posssess this α-1,6-linked fucose, is no longer retarded on VFA and PSA or retained on insolubilized LCA. This shows the importance of the N-acetyl glucosamine-asparagine sequence in the binding reaction and this can be related to the fact that the chitobiose core gives to the whole glycan a more rigid conformation as opposed to the random configuration of the oligosaccharide obtained by hydrazinolysis.

These interactions between glycopeptides such as GP-h-LTF and LCA can also involve charge effects and (or) some hydrophobic interactions between an hydrophobic area in or near the carbohydrate binding site of the lectin and aromatic residues of the peptidic part of the glycopeptides or glycoproteins.

These results on the specificities of LCA and PSA were recently confirmed and extended by Kornfeld *et al.,* (1981), who have shown that LCA or PSA can interact also with tri-antennary glycopeptides of the N-acetyllactosaminic type, possessing an α-L-fucose residue substituting in C-6 position the N-acetylglucosamine residue involved in the N-glycosylamine bond, but an important condition for this interaction to take place is that this third branch substitutes a core α-mannose residue in the C-6 position. If the substitution is in C-4 position, LCA or PSA do not show any affinity for these new tri-antennary structures.

Conclusion

Our results show that four lectins (Con A, LCA, VFA and PSA), considered to be identical in terms of α-D-mannose or glucose specificity, possess the ability to

recognize fine differences in more complex carbohydrate structures. While insolubilized Con A represents a very useful tool for the fractionation of both N-acetyllactosaminic and oligomannosidic type glycopeptides or related oligosaccharides, the three others lectins will also be very useful for the separation of N-acetyllactosaminic type glycopeptides on the condition that an α-L-fucose residue substitutes in C-6 position the N-acetylglucosamine residue involved in the N- glycosylamine bond.

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