The fine sugar specificity of the *Lathyrus ochrus* seed lectin and isolectins

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Received 4 August 1984

Glycoproteins and their derived glycopeptides have been used to define the specificity of the *Lathyrus ochrus* lectin and isolectins, by determining their ability to inhibit the agglutination of human erythrocytes induced by the lectin or isolectins. This α-D-mannose/α-D-glucose-specific lectin possessess the ability to recognize a well-defined saccharide sequence on a bi-antennary *N*-acetyllactosamine-type glycan. For other *Vicieae* lectins, the best inhibitor is a glycopeptide from human lactotransferrin with an α-L-fucose residue at the C-6 position on the *N*-acetylglucosamine residue involved in the *N*-glycosylamine bond. This fucose seems to be a major determinant of the binding since its removal with an α-L-fucosidase gives glycopeptides 8-fold less inhibitory. Our results confirm that the *Vicieae* lectins are evolutionarily related proteins even at the level of their binding sites.

Lectin *Isolectin* *Sugar specificity* Lathyrus ochrus

1. INTRODUCTION

Recently, it has been shown that 4 lectins considered 'identical' in terms of α-D-mannose and α-D-glucose specificity, i.e., Con-A and lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and broad bean (*Vicia faba* L.) lectins, possess the ability to recognize fine differences in more complex structures belonging to glycoproteins with *N*-glycosidic linkage [1]. A clear distinction can be made between Con-A and the 3 other lectins for which the presence of an α-(1-6)-linked fucose appears as a major determinant for a complete recognition. Such a difference of specificity allowed the use of these immobilized lectins for fractionation of both *N*-acetyllactosamine- and oligomannosidic-type glycopeptides [2–4].

Here, various glycoproteins and their derived glycopeptides have been used to define the specificity of another α-D-mannose/α-D-glucose specific lectin isolated from *Lathyrus ochrus* seeds, by determining their ability to inhibit the agglutination of human erythrocytes induced by the lectin.

2. MATERIALS AND METHODS

2.1. *Isolation of the* L. ochrus *lectin and isolectins*

The seeds of *L. ochrus* were obtained from plants cultivated under field conditions.

The isolation of the *L. ochrus* lectin and isolectins has been described elsewhere [18]. Briefly, LoL was isolated from seed flour with 50 mM Tris (pH 7.6), 0.15 M NaCl and the protein precipitated at 30–60% $(NH_4)_2SO_4$ were applied to a Sephadex G-100 column equilibrated with the

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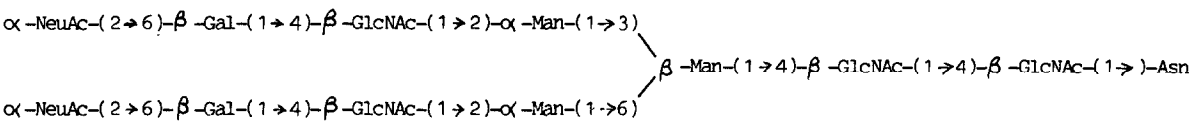
Abbreviations: LoL, *Lathyrus ochrus* lectin; LoLI, *Lathyrus ochrus* isolectin I; LoLII, *Lathyrus ochrus* isolectin II; Con-A, Concanavalin A; PBS, 0.1 M phosphate-buffered saline (pH 7.2)

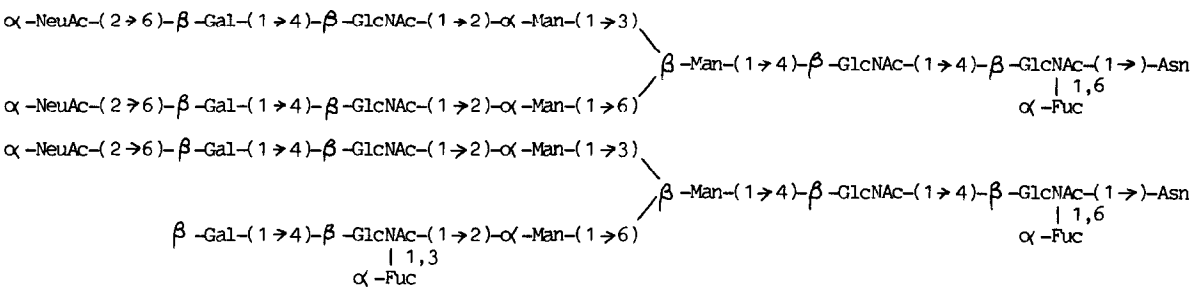
Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

Fig. 1. Structure of the main glycans found in the glycoproteins or derived glycopeptides used in the present study

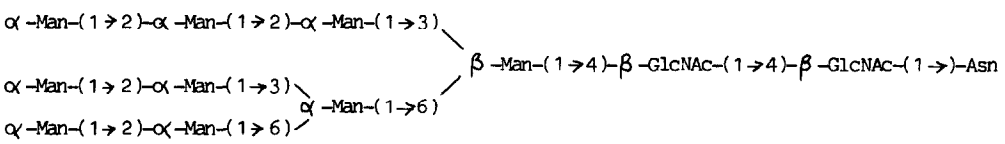
A. Human serotransferrin :



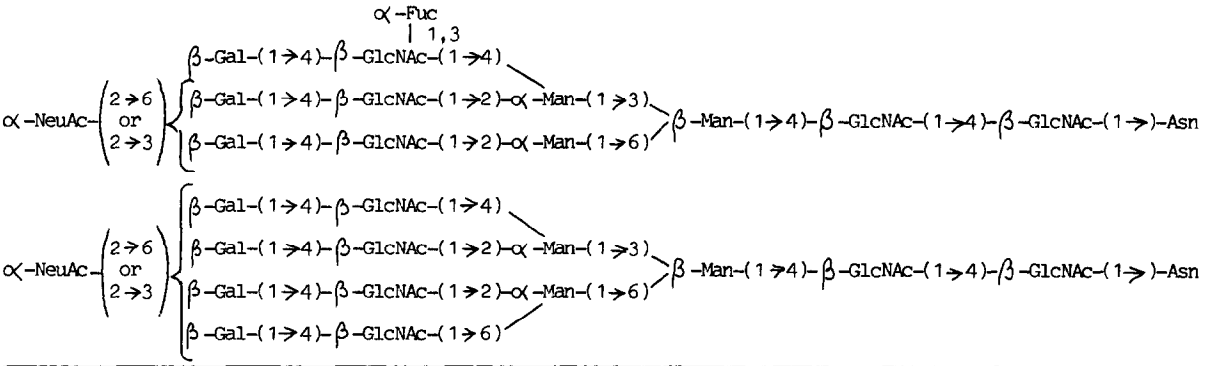
B. Human lactotransferrin :



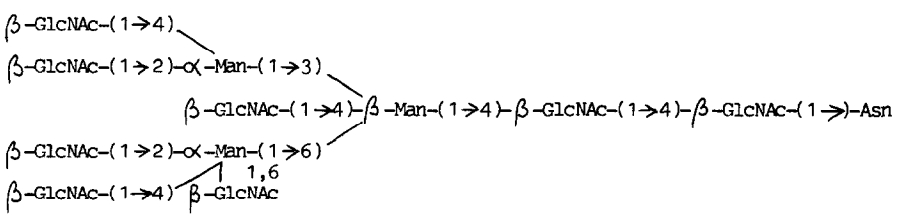
C. Bovine lactotransferrin :



D. Human α1-acid glycoprotein :



E. Hen ovomucoid :



same buffer. The retained lectin was subsequently eluted with 0.1 M glucose.

The separation of the two isolectins was achieved by chromatofocusing of the whole LoL on a PBE 94 (Pharmacia) column over pH 8.4–5.0. Two main fractions corresponding respectively to LoLI (pI 7.2) and LoLII (pI 6.0) were obtained. The purity of the two isolated isolectins was checked by polyacrylamide gel electrophoresis (PAGE) [5] and analytical isoelectric focusing on polyacrylamide gel slab over pH 8.0–5.0.

2.2. *Origin of glycoproteins and glycopeptides*

Human transferrin, human lactotransferrin and their derived glycopeptides, as well as the glycopeptide of the oligomannosidic-type isolated from bovine lactotransferrin, were gifts of Professor G. Spik.

Human α_1-acid glycoprotein was isolated according to [6] modified by introduction of a CM-trisacryl purification step according to [7]. Hen ovomucoid was isolated according to [8]. Glycopeptides derived from these two glycoproteins were prepared by extensive pronase digestion as described in [9].

The different glycans present in the above mentioned glycoproteins and related glycopeptides are shown in fig.1. They belong to two types of glycan structures: the *N*-acetyllactosamine type and the oligomannoside type, as proposed in [10].

2.3. *Haemagglutination test*

Agglutination of human ORh$^+$ erythrocytes by *L. ochrus* lectin and isolectins and inhibition of haemagglutination by various glycoproteins and glycopeptides were carried out in PBS in Linbro microtiter U-plates Titertek (Linbro Scientific Co., Hamden, USA), as described [1].

Results were expressed as the minimum concentration (mM) of glycoproteins or glycopeptides required to completely inhibit 4 haemagglutination doses. Account was taken of the 3-fold dilution caused by the addition of lectin/isolectin and erythrocytes.

2.4. *Protein analysis*

Protein concentration was estimated according to [11] using bovine serum albumin (Sigma) as standard.

3. RESULTS

LoL purified by affinity chromatography on Sephadex G-100 and the two derived isolectins LoLI and LoLII fractionated by subsequent chromatofocusing, possess about the same haemagglutinating activity towards human ORh$^+$ erythrocytes: the minimum protein concentrations producing complete haemagglutination are, respectively, 15.75 μg/ml for the whole lectin, 21.9 μg/ml for LoLI and 17.6 μg/ml for LoLII.

Table 1

Minimum concentration (mM) of sugar giving complete inhibition of haemagglutination

Compounds	LoL	LoLI	LoLII
D-Mannose	3.75	3.75	3.75
Human serotransferrin	>0.033	>0.033	>0.033
Human lactotransferrin	0.0041	0.0082	0.0041
Glycopeptide from human serotransferrin	0.103	0.0515	0.0515
Glycopeptide from human lactotransferrin	0.0125	0.0125	0.0125
Glycopeptide from bovine lactotransferrin	1.66	0.83	0.83
Human α_1-acid glycoprotein	>0.033	>0.033	>0.033
Hen ovomucoid	>0.033	>0.033	>0.033
Glycopeptide from hen ovomucoid	>1.66	>1.66	>1.66

Data from table 1 clearly show that both LoLI and LoLII exhibit an identical sugar specificity. In addition, human lactotransferrin and derived glycopeptides appear as the best inhibitors for the two isolectins as well as for the whole lectin. However, removal of all the fucose residues from these glycopeptides with an α-L-fucosidase from rat kidney gives glycopeptides 8- to 4-fold less inhibitory, as are the glycopeptides isolated from human serotransferrin. These results underline the importance of the α-(1-6)-linked fucose as a major determinant of the binding.

The glycopeptide isolated from bovine lactotransferrin, which possesses the pentasaccharide core substituted by additional α-linked mannose residues (oligomannose-type structure), is 132- and 16-fold less inhibitory, respectively, than glycopeptides isolated from human lactotransferrin and human serotransferrin. These findings clearly indicate that both LoL and the isolectins exhibit very little affinity for oligomannose-type structures.

4. DISCUSSION

LoL, composed of two closely related isolectins LoLI and LoLII, appears to be another example of α-D-mannose/α-D-glucose-specific lectin which possesses the ability to recognize precisely determinants on a complex bi-antennary *N*-acetyllactosamine-type glycan. As for broad bean, lentil and pea lectins, the α-L-fucose residue which substitutes in C-6 the *N*-acetylglucosamine residue involved in the *N*-glycosylamine bond, is a major determinant of the binding.

Recently, authors in [12] postulated that a lectin isolated from sweet pea (*Lathyrus odoratus*) seeds, might also require such an α-L-fucose residue for interaction with the complex carbohydrate moiety of human ceruloplasmin. On the basis of our present results, we may predict that the recently purified lectin from *Lathyrus tingitanus* seeds [13] together with the *Vicia hirsuta* lectin I [14], both of which exhibit an α-D-mannose/α-D-glucose specificity, will also present a similar fine specificity towards *N*-acetyllactosamine-type glycans with this L-fucose residue linked to the core *N*-acetylglucosamine.

Interestingly, all the above mentioned species belong to the tribe of *Vicieae* which includes the 4 main lectin-containing genera: *Lathyrus, Lens, Pisum* and *Vicia*. Their lectins are two-chain lectins which are made of light (α) and heavy (β) subunits and possess an α2β2 structure. The only known exception concerns *Vicia cracca* which remarkably contains lectins belonging to the two- and one-chain groups [15]. In addition, the amino acid sequences homologies [16] and the immunochemical crossreactions [17] demonstrated between various lectins from the *Vicieae* tribe, strongly suggest that lectins from the *Vicieae* are evolutionarily related and conserved proteins. Moreover, our results suggest that relationships between the *Vicieae* lectins have also been conserved at the level of their binding sites, during evolution, since they exhibit a very similar quite fine specificity towards complex glycan structures. Thus, Con-A, which has a similar α-D-mannose/α-D-glucose specificity but is built up of 4 identical subunits (one-chain group lectin) and which belongs to another Leguminosae tribe, does not react with the same precise glycan structures recognized by the *Vicieae* lectins.

ACKNOWLEDGEMENTS

This work was supported in part by the Centre National de la Recherche Scientifique (LA no.217: Relation structure-fonction des constituants membranaires; Dr Pr.J. Montreuil), by the Institut National de la Santé et de la Recherche Médicale (contracts 134.012 and 832.029) and by the Ministère de l'Industrie et de la Recherche (contract 82-L-1099). The authors are very grateful to Dr G. Spik for providing human transferrin and lactotransferrin, as well as their derived glycopeptides and for the glycopeptide isolated from bovine lactotransferrin.

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