*Glycoconjugate J (1987) 4: 371-378*

# Structure-function Relationships Among *Lathyrus* Lectins

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Received May 27, 1987.

*Key words: Lathyrus lectins/isolectins, structure-function relationship, amino acid sequences, blood group specificity, inhibition by sugars, interaction with human serum glycoproteins, mitogenicity*

**The structure-function relationship of eight structurally very similar *Lathyrus* lectins, which are two-chain lectins from the *Vicieae* tribe, has been studied. Hemagglutination activity, inhibition of hemagglutination, glycoprotein reactivity and mitogenic activity were determined for each of the lectins. Despite the close structural resemblance, marked differences in the carbohydrate binding activity of the *Lathyrus* lectins were recorded. These functional differences were related to small conformational changes due to minor differences in amino acid sequence of each *Lathyrus* lectin.**

Despite the widespread use of lectins for the analysis of free or membrane-bound glycoconjugates and the increasing amount of information available on lectin composition and specificity, little is known to date on the structure-function relationships of these intriguing molecules. Without any doubt, such a situation arises from the growing gap between the very limited number of lectins whose complete amino acid sequence and three-dimensional structure have been so far characterized and the multiplicity of these proteins for which the broad sugar specificity is now well known [1]. Apart from previous studies carried out on Concanavalin A (Con A) [2-4] and recently refined by Carver *et al.* [5], other reports have only been concerned with the ability of simple sugars or more complex glycans to bind to the carbohydrate binding sites of various lectins [6]. However, Olsen [7] has shown that other single- and two-chain legume lectins are strongly predicted to contain three-dimensional structures very similar to that of Con A, in which three distinct domains are involved. Thus, a comparison of the complete amino acid sequence of a given legume lectin with Con A, allows a facile identification of the amino acids in homologous positions to those implicated in the carbohydrate binding sites of the lectin. In addition, it is possible to

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detect amino acids involved in the two other functional features of the Con A protomer: the bivalent cation binding sites and the hydrophobic cavity, which are believed to be also present [8, 9] in other legume lectins. The observed changes, together with those of various functional properties (e.g. the binding of simple sugars), could in turn give an insight into the structure-function relationships of the lectin. We present here data concerning some *Lathyrus* lectins, which are two-chain lectins [10] from the *Vicieae* tribe, exhibiting a broad sugar specificity very similar to that of Con A (inhibition by glucose/mannose and derivatives).

## Materials and Methods

### *Plant Material*

The seeds of *Lathyrus aphaca* L., *L. articulatus* L., *L. cicera* L., *L. hirsutus* L., *L. ochrus* (L.) DC., *L. odoratus* L., *L. tingitanus* L., *L. vernus* Bernh. and *Pisum sativum* L., var. petit provençal nain, were harvested from plants cultivated under field conditions. *Canavalia ensiformis* DC. seed meal was purchased from Serva Finebiochemica (Heidelberg, W. Germany) for Con A isolation.

### *Isolation of Lectins*

All lectins were isolated by affinity chromatography on a Sephadex G-100 column, as previously described [11]. Briefly, seed flour was soaked overnight in 0.05 M Tris-HCl (pH 7.6), 0.15 M NaCl (Tris buffered saline), and the solubilized proteins were precipitated between 30-60% ammonium sulfate saturation. After extensive dialysis against Tris buffered saline, proteins were chromatographed on a Sephadex G-100 column equilibrated with the same buffer and elution of the retained lectins was performed by adding 0.1 M glucose to the eluting buffer. After precipitation at 90% ammonium sulfate saturation, lectins were solubilized in the minimum of buffer, dialysed, and stored frozen at -30°C until needed. The purity of these lectins was checked by PAGE at basic pH [12], the protein fractions being stained with Coomassie blue R-250 according to [13]. The concentration of lectin solutions was estimated colorimetrically by the microbiuret method of Goa [14], using bovine serum albumin (Sigma, St. Louis, MO, USA) as standard.

### *Hemagglutination Techniques*

The hemagglutinating activity of lectins toward red blood cells (RBC) of various ABO and Rh groups was checked by two-fold serial dilution in 0.1 M sodium phosphate (pH 7.2) buffered 0.15 M NaCl (PBS). Crude lectin solutions obtained by soaking 1 g seed meal overnight in 5 ml 0.05 M Tris-HCl (pH 7.6), 0.15 M NaCl, and subsequent centrifugation, were used throughout. To each lectin solution (200 $\mu$l), 200 $\mu$l of a 1% suspension of thrice-washed human RBC of different ABO and Rh groups in PBS was added and agglutination was estimated 12 h later. Inhibition of hemagglutination by simple sugars was also tested by serial two-fold dilution, but in 50 $\mu$l and by using purified lectin solutions, as described elsewhere [15]. Briefly, to each sugar solution, 50 $\mu$l of lectin solution containing 37.5 $\mu$g of lectin/ml was added and, after standing for 1 h, 200 $\mu$l of thrice-washed human RBC (O Rh+) was added. As above, agglutination was estimated 12 h later. Human RBC of various ABO and Rh phenotypes were obtained from healthy blood donors.

**Table 1.** Hemagglutinating activity of various crude lectin solutions towards red blood cells of different ABO and Rh phenotypes (activity is expressed as titer which corresponds to the reciprocal of the last twofold dilution still giving complete hemagglutination). Concanavalin A (Con A) and lectins from *Lathyrus aphaca* (L.aph.), *L. articulatus* (L.art.), *Lathyrus cicera* (L.cic.), *L. hirsutus* (L.hir.), *L. ochrus* (L.och.), *L. odoratus* (L.odo.), *L. tingitanus* (L.tin.), *L. vernus* (L.ver.) and *Pisum sativum* (P.sat.), have been used as lectin source.

| Blood group | Lectins from | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| phenotypes | Con A | L.aph. | L.art. | L.cic. | L.hir. | L.och. | L.odo. | L.tin. | L.ver. | P.sat |
| $A_1$ RH+ | 128 | 64 | 128 | 16 | 32 | 128 | 16 | 128 | 64 | 64 |
| $A_1$ Rh− | 128 | 128 | 128 | 16 | 32 | 256 | 32 | 128 | 64 | 64 |
| $A_2$ Rh+ | 128 | 128 | 64 | 16 | 32 | 128 | 32 | 128 | 64 | 128 |
| $A_2$ Rh− | 256 | 128 | 64 | 32 | 64 | 128 | 64 | 256 | 128 | 128 |
| B Rh+ | 128 | 128 | 128 | 16 | 32 | 128 | 32 | 128 | 64 | 64 |
| B Rh− | 256 | 128 | 128 | 32 | 64 | 128 | 64 | 256 | 64 | 128 |
| $A_1$B Rh+ | 256 | 128 | 128 | 16 | 32 | 256 | 32 | 128 | 128 | 64 |
| $A_1$B Rh− | 128 | 128 | 128 | 32 | 64 | 128 | 32 | 256 | 64 | 64 |
| $A_2$B Rh+ | 128 | 128 | 128 | 32 | 64 | 256 | 32 | 256 | 64 | 64 |
| $A_2$B Rh− | 128 | 128 | 128 | 16 | 32 | 128 | 32 | 128 | 128 | 128 |
| O Rh+ | 256 | 128 | 128 | 32 | 64 | 256 | 64 | 256 | 128 | 64 |
| O Rh− | 128 | 128 | 128 | 16 | 32 | 128 | 64 | 256 | 64 | 64 |

*Precipitation of Human Serum Glycoproteins by Lectins*

A pool of sera from healthy blood donors was chromatographed [15] on columns of CNBr-Sepharose 4B (Pharmacia, Uppsala, Sweden) covalently coupled to lectins and the retained serum glycoproteins were subsequently eluted by adding 0.1 M glucose to the eluting buffer (0.05 M Tris-HCl, pH 7.6; 0.15 M NaCl). Glucose was eliminated by extensive dialysis against the same buffer and glycoproteins were identified by double immunodiffusion on agarose gels [16], with specific antisera (Calbiochem, San Diego, CA, USA) against the main human serum glycoproteins, as previously described [17].

*Mitogenic Activity of Lectins*

Human peripheral blood mononuclear cells were isolated from healthy donors by Ficoll-Paque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. As previously reported [18], activation of the lymphocytes was measured by incorporation of [methyl-$^3$H]thymidine (5.0 Ci/nmol, Amersham International, Amersham, UK) into the DNA. Lymphocytes ($2 \times 10^5$) in RPMI 1640 supplemented with 4 mM L-glutamine, streptomycin (50 $\mu$g/ml), penicillin (50 IU/ml), 1% (v/v) 100× non-essential amino acids and 10% (v/v) fetal calf serum (Flow Laboratories, Ayrshire, UK), were incubated for 65 h in microtiter plates in the presence of each lectin. The final volume was 100 $\mu$l and 12 h before harvest the cells were pulsed with 1 $\mu$Ci [methyl-$^3$H]thymidine/well. When the lectin-induced lymphocyte activation was performed under serum-free conditions, 0.2% methyl cellulose (Methocel A4M, Dow Chemicals, Stade-Brunhausen, W. Germany), prepared as described previously [19], was used instead of fetal calf serum.

**Table 2.** Minimum concentration (mM) of sugar giving complete inhibition of hemagglutination. The lectin concentrations used were 37.5 μg/ml, these concentrations being 4-8 times higher than that producing complete hemagglutination in the last twofold dilution. The following sugars were not inhibitory at a final concentration of 200 mM: arabinose, fucose, galactose, galactosamine, *N*-acetyl-D-galactosamine, α-methyl-D-galactoside, β-methyl-D-galactoside, β-methyl-D-mannoside, L-rhamnose, ribose. For abbreviations used for lectins see Table 1.

| Sugar | Lectins from | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Con A | L.aph. | L.art. | L.cic. | L.hir. | L.och. | L.odo. | L.tin. | L.ver. | P.sat |
| Mannose | 3.12 | 6.25 | 6.25 | 6.25 | 3.12 | 6.25 | 3.12 | 6.25 | 3.12 | 3.12 |
| Glucose | 12.5 | 25 | 25 | 25 | 6.25 | 12.5 | 6.25 | 12.5 | 6.25 | 12.5 |
| Fructose | 6.25 | 50 | 50 | 25 | 12.5 | 25 | 12.5 | 25 | 12.5 | 12.5 |
| Glucosamine | 25 | 100 | 50 | 50 | 25 | 100 | 25 | 50 | 25 | 25 |
| α-Methyl-mannoside | 0.39 | 3.12 | 3.12 | 3.12 | 0.78 | 3.12 | 0.78 | 1.56 | 0.78 | 1.56 |
| α-Methyl-glucoside | 1.56 | 12.5 | 12.5 | 12.5 | 3.12 | 12.5 | 3.12 | 12.5 | 6.25 | 6.25 |
| *N*-Acetyl-D-glucosamine | 12.5 | 25 | 25 | 25 | 6.25 | 25 | 12.5 | 12.5 | 6.25 | 12.5 |
| Sucrose | 12.5 | 25 | 12.5 | 12.5 | 6.25 | 12.5 | 12.5 | 12.5 | 6.25 | 12.5 |

**Table 3.** List of the main human serum glycoproteins interacting (+) with various lectins.

| Human serum | Lectins from | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| glycoproteins | Con A | L.aph. | L.art. | L.cic. | L.hir. | L.och. | L.odo. | L.tin. | L.ver. | P.sat |
| Orosomucoid | | | | + | | | | | | |
| α1-Antitrypsin | + | + | + | + | | | | | | + |
| α1B-Glycoprotein | | + | + | + | | + | + | + | + | + |
| α1-Antichymotrypsin | + | | | | | | | | | |
| α2HS-Glycoprotein | + | + | + | + | | + | + | + | + | + |
| C1-Inactivator | + | + | + | + | + | + | + | + | + | + |
| Gc-Globulin | + | | | | | | | | | + |
| Haptoglobin | + | + | + | + | + | + | + | + | + | + |
| α2-Macroglobulin | + | + | + | + | + | + | + | + | + | + |
| β-Lipoprotein | + | + | + | + | | | | + | | + |
| Ceruloplasmin | + | + | + | + | + | + | + | + | + | + |
| Hemopexin | + | + | + | + | + | + | + | + | + | + |
| Transferrin | + | + | + | + | + | + | + | + | + | + |
| C3-Component | | + | + | + | | | | + | | |
| IgA | + | + | + | + | + | + | + | + | + | + |
| IgG | + | + | + | + | + | + | + | + | + | + |
| IgM | + | + | + | + | + | + | + | + | + | + |

**Table 4.** Mitogenic doses (expressed as μg lectin/ml) yielding maximum cellular response measured in medium containing 10% fetal calf serum (R10) or under serum-free conditions in medium containing 0.2% methyl cellulose (RO).

| Lectins from | Lectin concentration (μg/ml) | |
|---|---|---|
| | R10 medium | R0 medium |
| *Con A* | 3 | 1 |
| *Lathyrus aphaca* | 30 | 10 |
| *Lathyrus articulatus* | 10 | 3 |
| *Lathyrus cicera* | 100 | 30 |
| *Lathyrus hirsutus* | 50 | 30 |
| *Lathyrus ochrus* | 3 | 3 |
| *Lathyrus odoratus* | 100 | 10 |
| *Lathyrus tingitanus* | 50 | 3-10 |
| *Lathyrus vernus* | 50 | 10-30 |

*Amino Acid Sequences and Conformations of Lectins*

The amino acid sequences of the assayed lectins were taken from [20-30]. The computerized method of Rose *et al.* [31] was employed to predict the exposed and buried regions along the constituent polypeptidic chains of the lectins.

## Results and Discussion

Although all the lectins studied could react with all of the human RBC exhibiting various ABO and Rh phenotypes (Table 1), some differences occurred among their hemagglutinating activities. Similarly, more or less marked differences appeared among the assayed lectins on comparing their hemagglutination inhibition by various simple sugars and sugar derivatives (Table 2), their ability to interact with human serum glycoproteins (Table 3) and their mitogenic activity toward human lymphocytes (Table 4). Such discrepancies could be explained on the basis of slight differences in the conformation of their carbohydrate binding sites. In this respect, a comparison of the amino acid sequences of these lectins (Fig. 1), clearly shows that a high degree of homology exists among them. In addition, most of the amino acids involved in the three main structural features of the Con A protomer, i.e. the hydrophobic cavity [2], the bivalent cation binding sites [2, 32] and the carbohydrate binding site [2-5], are well conserved in other lectins. Moreover, the secondary structures of the *Lathyrus* lectins, predicted according to the computerized methods of Chou and Fasman [33] and Garnier *et al.* [34], appear to be very similar [35]. Accordingly, the exposed and buried regions of these lectin polypeptidic chains estimated by both the Kyte and Doolittle [35] and the Rose *et al.* (Fig. 2) methods, are readily superimposed. All of these results suggest that Con A and the two-chain legume lectins from lentil, pea and *Lathyrus* species, possess a quite comparable three-dimensional conformation, that is consistent with the Olsen model [7]. The previous findings of Debray *et al.* [36-38] on the interaction between Con A or other two-chain lectins and various complex glycans, are in complete

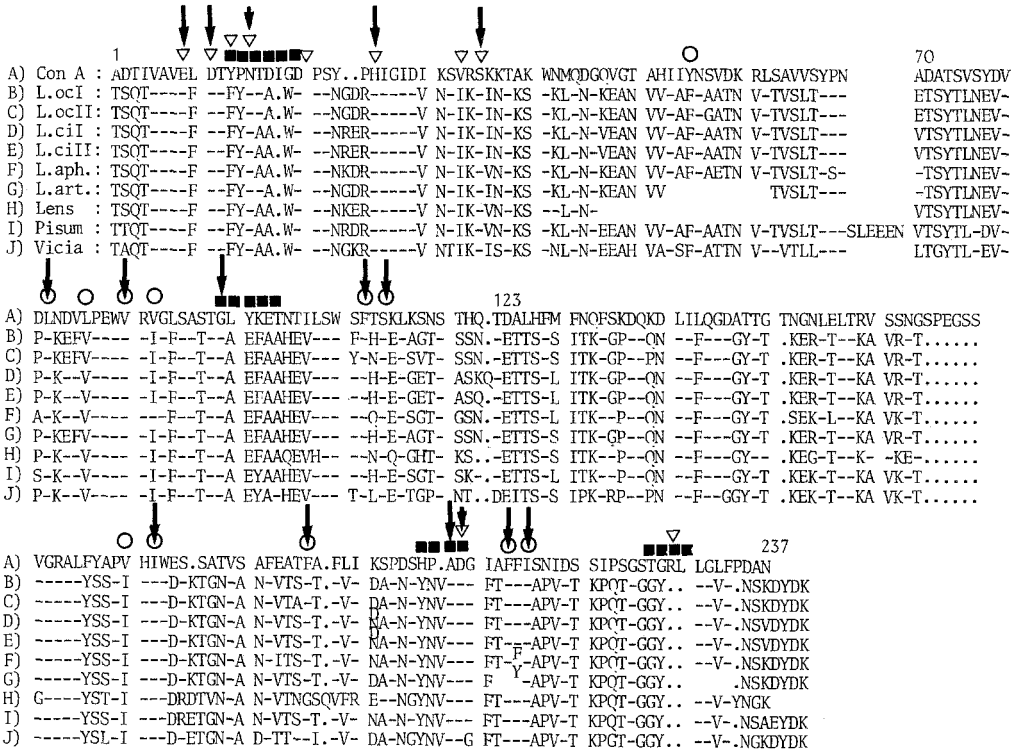


**Figure 1.** Comparison of the amino acid sequences of Con A and various two-chain lectins. The amino acids involved in the carbohydrate binding site (■), the bivalent cation binding sites (△) and the hydrophobic cavity (○) of Con A have been indicated. Arrows indicate invariant residues involved in these functional features of the Con A protomer (... deletions introduced to maximize homologies).

agreement with the above reported results: their findings clearly show that lectins possessing the same broad sugar specificity toward simple sugars, could in fact exhibit different fine sugar specificities toward complex glycans. Therefore, the results indicate that the differences observed among *Lathyrus* lectins in their affinity toward complex glycans could be related to small changes in the conformation of their carbohydrate binding sites. These conformational changes have to be regarded as a consequence of the slight differences observed among the amino acid sequences of their constituent light and heavy subunits, which only differ in a few positions. However, these differences are slight enough to maintain an almost identical general overall conformation, that could explain why two-chain lectins, like Con A, have retained throughout evolution the ability to bind bivalent cations and to interact with hydrophobic compounds such as plant hormones [8, 39, 40]. Assuming that one or all of the functional features (carbohydrate binding site, cation binding sites and hydrophobic cavity) of Con A and other two-chain lectins are necessary to allow them to fulfil their role(s) in plants, the structure-function relationships of these proteins would thus reflect the limited

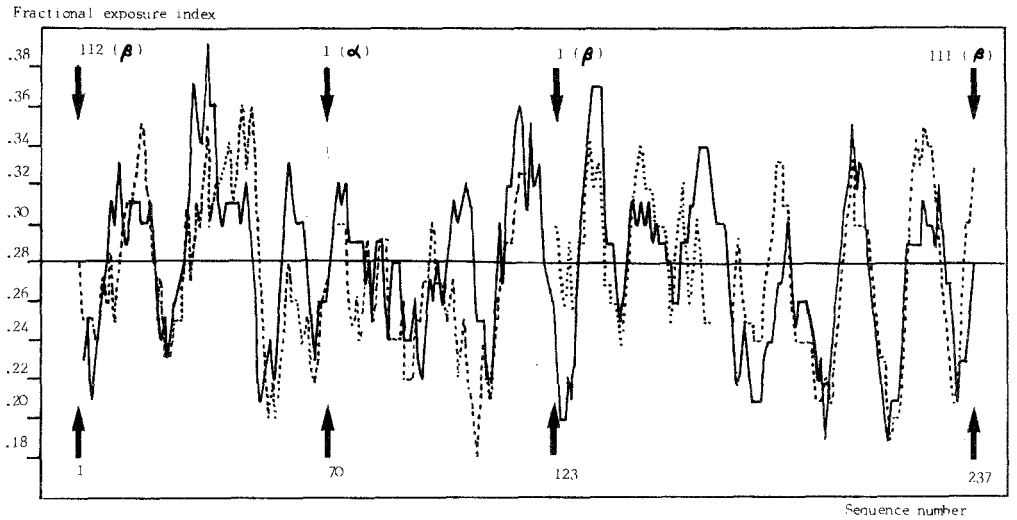


**Figure 2.** Comparison of the predicted exposed and buried regions of Con A (—) and *Lathyrus ochrus* isolectin I (----). The solvent exposure index of Rose *et al.* [31] at each residue, taken as a running average with the preceding and following three residues, has been plotted for the common aligned residues of the two lectins. Due to the circular permuted sequence homology existing between Con A and other two-chain lectins [41], residues 1, 70, 123 and 237 of Con A (lower arrows) correspond respectively to residues 112 (heavy subunit), 1 (light subunit), 1 (heavy subunit) and 111 (heavy subunit) of the *L. ochrus* isolectin ((upper arrows). Maxima and minima correspond respectively to predicted exposed and buried regions of lectins. The horizontal line which nearly coincides with the 0.28 fractional exposure index, corresponds to the overall average of the two common sequences.

changes that very conserved molecules have undergone during evolution. Other experiments dealing with the interaction of *Lathyrus* lectins with more or less complex glycans are now in progress in order to verify such a structure-function relationship based upon the slight conformational changes of these molecules throughout the speciation process of the genus *Lathyrus*.

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