Subunit Structure of *Vicia graminea* Anti-(Blood-Group N) Lectin

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The subunit of the *Vicia graminea* lectin with blood-group-N specificity was examined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and gel filtration in 6 M-guanidinium chloride, and its molecular weight was found to be 25 000. The unique N-terminal sequence of the first nine residues of the lectin confirmed that *Vicia* lectin consists of four identical chains non-covalently linked. Finally the micro-heterogeneity of the lectin shown by analytical isoelectric focusing is discussed.

Lectins constitute a group of cell-agglutinating proteins that are generally found in plants, particularly among the Leguminosae, in lower invertebrates and even in some higher animals (Sharon & Lis, 1972; Lis & Sharon, 1977). The biological characterization of some commonly used lectins has led to the belief that their multivalent binding ability is attributable to their subunit structure (Lis & Sharon, 1973). The native molecule can be either a homopolymer composed of identical subunits [*Arachis hypogaea* (peanut) (Foriers *et al.*, 1977) and *Canavalia ensiformis* (jack-bean) (concanavalin A; Abe *et al.*, 1971) lectins] or a heteropolymer made up of different molecular species as subunits [*Ricinus communis* (castor-bean) (Li *et al.*, 1975) and *Phaseolus vulgaris* (kidney-bean) (phytohaemagglutinin; Miller *et al.*, 1975) lectins]. Lectins specific for blood groups may also be either homopolymers, e.g. *Bandeiraea simplicifolia* (Hayes & Goldstein, 1973), which specifically agglutinates human blood-group-B erythrocytes, or heteropolymers, like the *Dolichos biflorus* (horse-gram) (Carter & Etzler, 1975) lectin, which selectively acts on blood-group-A1 erythrocytes. To date, only one lectin specific for MN blood groups has been purified and characterized (Prigent & Bourrillon, 1976). This is the *Vicia graminea* lectin, which is specific for the N blood group and has a mol.wt. of 106 000. In the present paper we report that this lectin is a tetramer with a single type of subunit.

Materials and Methods

Isolation of the lectin

Vicia graminea lectin was isolated from seed extracts as previously described (Prigent &

Bourrillon, 1976) and its homogeneity demonstrated by polyacrylamide-gel electrophoresis, immunoelectrophoresis and analytical ultracentrifugation.

Gel filtration

The molecular weight of the subunit was estimated by gel filtration on a column (3 cm × 95 cm) of Sephadex G-150 equilibrated with 6 M-guanidinium chloride and 0.1% β -mercaptoethanol in 0.2 M-phosphate buffer, pH 7. The column was calibrated as described by Fish *et al.* (1969) and operated at room temperature. Fractions (2 ml) were collected at a flow rate of 6 ml/h. Before application of the lectin, a mixture of molecular-weight markers was run [5 mg of *Vicia graminea* lectin, 10 mg of lysozyme (mol.wt. 13 000), trypsin (23 000), subunits of concanavalin A (27 000) and *Robinia pseudo-acacia* (black-locust) lectin (30 000), ovalbumin (45 000)]. These markers were dialysed against the elution buffer for 48 h.

Polyacrylamide-gel electrophoresis

Disc-gel electrophoresis was carried out as described by Rodbard & Chrambach (1971) in a 7% (w/v) polyacrylamide gel at pH 10.2. In some cases electrophoresis was also performed in slabs of a 3–24% gradient of polyacrylamide at pH 8.6, as described by Margolis & Kenrich (1967). SDS/polyacrylamide-gel (10%) electrophoresis was used to determine the molecular weight of the subunit, as described by Weber & Osborn (1969). Protein samples were heated for 5 min at 100°C in 10 mM-sodium phosphate buffer, pH 7, containing 1% SDS, with or without 1% β -mercaptoethanol. Bovine serum albumin (mol.wt. 67 000), ovalbumin, subunit of concanavalin A and cytochrome *c* (mol.wt. 12 400) were used as fully reduced reference

Abbreviation used: SDS, sodium dodecyl sulphate.

proteins and 25 μ g of each protein was applied to the top of the gel. Electrophoretic mobilities were calculated after staining with Coomassie Blue.

Isoelectric focusing

Analytical isoelectric focusing was carried out as described by Doerr & Chrambach (1971) in 5% polyacrylamide gels containing 2% Ampholines (pH 4–6; LKB). Protein samples were stained with Coomassie Blue.

Amino acid-sequence determination

Vicia graminea lectin (200 nmol) was sequentially degraded in a Beckman sequencer, model 890 C, by the method of Edman & Begg (1967), with the *NN'*-dimethylbenzylamine program as modified by Hermodson *et al.* (1972). The resulting amino acid phenylthiohydantoin derivatives were identified by t.l.c. in chloroform/methanol (99:1 and 22:3, v/v), and by high-pressure liquid chromatography.

Results and Discussion

It has been demonstrated, both by high-speed sedimentation equilibrium and by gel filtration on Sephadex G-150, that the molecular weight of native *Vicia graminea* lectin is 106 000. In the same study an apparent mol.wt. of 25 000 ($\pm 10\%$) was obtained by SDS/polyacrylamide-gel electrophoresis of the lectin (Prigent & Bourrillon, 1976).

When the lectin was subjected to 6 M-guanidinium chloride denaturation before gel filtration on a column of Sephadex G-150 in a dissociating medium, a single symmetrical peak was obtained. A mol.wt. of $26\,000 \pm 5\%$ was calculated for this subunit from the partition coefficients (K_D). Comparison of the molecular weights of the native molecule and the subunit suggest that *Vicia graminea* lectin is composed of four similar subunits. The fact that the subunits are dissociated by SDS irrespective of the presence of a reducing agent indicates that the subunits are held together by non-covalent bonds (Figs. 1a and 1b). The *Vicia graminea* lectin therefore has the same subunit structure as that observed in other well-characterized lectins, whether or not they are specific for blood groups (Bourrillon, 1976). The subunit structure of lectin may play a role in determining the biological, and particularly the agglutinating, properties of these substances, as has been proved in the case of *Ricinus communis* haemagglutinin (Funatsu *et al.*, 1977). The presence of a single type of subunit, as indicated by SDS/polyacrylamide-gel electrophoresis, was confirmed by the determination of the partial *N*-terminal sequence of the lectin. Analysis showed only isoleucine in the *N*-terminal position. The isoleucine yield during this first step indicated that there was only one isoleucine residue per subunit. Determination of the first nine amino acids showed a single *N*-terminal sequence (Table 1), no microheterogeneity being noted for these first nine residues.

Fig. 1. Electrophoretic characteristics of *Vicia graminea* lectin

Polyacrylamide-gel electrophoresis was carried out in the presence of SDS with (b) or without (a) β -mercaptoethanol. (c) Disc-gel electrophoresis in 7% polyacrylamide was performed with 50 μ g of lectin at pH 10.2. (d) Electrophoresis at pH 8.6 in a gradient of polyacrylamide from 3% (top) to 24% (bottom) with 40 μ g of lectin. (e) Isoelectric focusing in a pH 6(top)–pH 4(bottom) gradient.

Table 1. Comparison of the *N*-terminal sequences (first nine residues) of the *Vicia graminea* agglutinin (as determined by the automated Edman technique) with those of other lectins: concanavalin A (Doerr & Chrambach, 1971), the *Ricinus communis* lectin (ricin) A- and B-chains (Edman & Begg, 1967) and the L-subunit of the *Phaseolus vulgaris* lectin (L-PHA) (Fish *et al.*, 1969)

Lectin	Residue no.	1	2	3	4	5	6	7	8	9	10
<i>Vicia graminea</i>		Ile	Asp	Tyr	Ile	Phe	Gln	Gly	Asp	Ala	—
L-PHA		Ser	Asn	Asp	Ile	Tyr	Phe	Asn	Phe	Glu	Arg
Concanavalin A		Ala	Asp	Thr	Ile	Val	Ala	Val	Glu	Leu	Asp
Ricin B-subunit		Ala	Asp	Val	Thr	Gln	Asp	Pro	Glu	Pro	Ile
Ricin A-subunit		Ile	Phe	Pro	Lys	Met	Tyr	Pro	Ile	Ile	Ile

It has been suggested that the genes providing the code for the various leguminous-plant lectins may have evolved from a common ancestral gene (Foriers *et al.*, 1977). We therefore compared the *N*-terminal sequence of the *Vicia graminea* lectin with those of other lectins (Table 1). The A-subunit of *Ricinus communis* lectin has the same *N*-terminal amino acid (isoleucine). The L-subunits of phytohaemagglutinin, concanavalin A and the B-subunit of *Ricinus communis* (castor-bean) lectin have aspartic acid in position-2, in common with the *Vicia graminea* lectin. The aromatic amino acids are represented in position-5: phenylalanine in the *Vicia graminea* sequence and tyrosine in that of phytohaemagglutinin. There are therefore only limited similarities between the *N*-terminal sequences of the different lectins.

When submitted to polyacrylamide-gel electrophoresis (Figs. 1c and 1d) at either pH 10.2 or 8.6, *Vicia graminea* lectin seemed homogeneous, but with isoelectric focusing on a pH 4–6 Ampholine gradient, four major bands were seen (Fig. 1e). Under such conditions, purified *Arachis hypogaea* lectin (M.-J. Prigent & R. Bourrillon, unpublished work), which is considered a homopolymer (Foriers *et al.*, 1977; Abe *et al.*, 1971), exhibits similar microheterogeneity and also yields four bands on isoelectric focusing. Other investigators have reported similar findings for concanavalin A and the purified lectin of *Glycine max* (soya bean) (Agrawal *et al.*, 1971; Lis *et al.* 1966).

Different explanations can be proposed for the apparent microheterogeneity observed on isoelectric focusing. Since our lectin has no sialic acid, the microheterogeneity cannot be attributed to different extents of sialylation, as has been observed for various animal glycoproteins (Zimmerman *et al.*, 1976). Since isoelectric focusing, even carried out in a denaturing medium, reveals different forms of the lectin, the possibility of variants arising from the polymerization of the molecule is excluded.

Chemical modification of the molecule during isolation or in the course of the isoelectric-focusing experiments could be considered, but these would seem unlikely, as the techniques used were 'gentle'. Multiple bands could also conceivably arise from heterogeneity in the oligosaccharide chain or from differences in the amide content of the asparagine or glutamine residues (Spencer & King, 1971). However, the most satisfactory explanation would seem to lie in the fact that the *Vicia graminea* seeds used were not selected on a genetic basis. Possibly the variants would be the products of several nearly identical genes. If there were slight differences between individual seeds, pooling of the seeds would result in the differences observed. Only determination of the complete primary sequence of the *Vicia graminea* lectin would provide the final answer.

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References

- Abe, Y., Iwabuchi, M. & Ishii, S. I. (1971) *Biochem. Biophys. Res. Commun.* **45**, 1271–1278
- Agrawal, B. B. L., Cuatrecasas, P. & Pollard, H. B. (1971) *J. Biochem. Biophys.* **124**, 218–229
- Bourrillon, R. (1976) *Biol. Med.* **4**, 31–52
- Carter, C. G. & Etzler, M. E. (1975) *Biochemistry* **14**, 2685–2689
- Doerr, P. & Chrambach, A. (1971) *Anal. Biochem.* **42**, 96–107
- Edman, P. & Begg, C. (1967) *Eur. J. Biochem.* **1**, 80–91

- Fish, W. W., Mann, R. G. & Tanford, C. (1969) *J. Biol. Chem.* **244**, 4989–4994
- Foiers, A., Wuilmart, C., Sharon, N. & Strosberg, A. D. (1977) *Biochem. Biophys. Res. Commun.* **75**, 980–986
- Funatsu, G., Ueno, S. & Funatsu, M. (1977) *Agric. Biol. Chem.* **41**, 171–174
- Hayes, C. E. & Goldstein, I. J. (1973) *J. Biol. Chem.* **249**, 1904–1914
- Hermanson, M. A., Ericsson, L. H., Titani, K., Neurath, H. & Walsh, K. A. (1972) *Biochemistry* **11**, 4493–4502
- Li, S. L., Wei, C. H., Lin, J. Y. & Tung, T. C. (1975) *Biochem. Biophys. Res. Commun.* **65**, 1191–1195
- Lis, H. & Sharon, N. (1973) *Annu. Rev. Biochem.* **42**, 541–574
- Lis, H. & Sharon, N. (1977) in *The Antigens*, vol. 4 (Sela, M., ed.), pp. 429–529, Academic Press, New York
- Lis, H., Friedman, C., Sharon, N. & Katchalski, E. (1966) *Arch. Biochem. Biophys.* **117**, 301–309
- Margolis, J. & Kenrich, K. G. (1967) *Biochem. Biophys. Res. Commun.* **27**, 68–73
- Miller, J. B., Hsu, R., Heinrikson, R. & Yachnin, S. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1388–1391
- Prigent, M.-J. & Bourrillon, R. (1976) *Biochim. Biophys. Acta* **420**, 112–121
- Rodbard, D. & Chrambach, A. (1971) *Anal. Biochem.* **40**, 95–134
- Sharon, N. & Lis, H. (1972) *Science* **177**, 949–959
- Spencer, E. M. & King, T. P. (1971) *J. Biol. Chem.* **246**, 201–208
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412
- Zimmerman, E. F., Bowen, D., Wilson, J. R. & Madapally, M. M. (1976) *Biochemistry* **15**, 5534–5543