

## Isolation and partial characterization of wheat-germ-agglutinin-like lectins from rye (*Secale cereale*) and barley (*Hordeum vulgare*) embryos

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(Received 29 October 1981/Accepted 14 December 1981)

Lectins have been isolated from embryos of *Secale cereale* (rye) and *Hordeum vulgare* (barley) by affinity chromatography on immobilized *N*-acetylglucosamine. Both lectins are dimeric proteins of two identical subunits of mol.wt. 18 000. They resemble strongly wheat-germ agglutinin with respect to their chemical, physical, biological and immunological properties.

Plant lectins are widely used as powerful tools in cell-surface and complex-carbohydrate research owing to their unique ability to bind specific sugars or sugar-containing macromolecules (Liener, 1976; Goldstein & Hayes, 1978). Among them, WGA takes an important place. This *N*-acetylglucosamine-specific lectin was found to produce several different effects on biological systems, all of which are apparently caused by interaction of the lectin with membrane components (Goldstein & Hayes, 1978). Extensive studies on WGA have led to a detailed characterization of its chemical, physical and biological properties (Allen *et al.*, 1973; Nagata & Burger, 1974; Privat *et al.*, 1974; Rice & Etzler, 1975; Wright, 1980*a,b*). An important question to be answered is whether this protein fulfils a physiological role, and, if so, what kind of role. Mirelman *et al.* (1975) demonstrated that WGA has an inhibitory effect on fungal growth, at least under conditions *in vitro*. It remains to be established, however, whether such an antifungal activity occurs also under natural conditions. In addition to this problem, the question can be put forward whether WGA is a unique lectin or just represents an example of a common type of what can be designated as 'cereal lectins'. A recent report on the isolation of a lectin from rice bran (Tsuda, 1979), which shares several characteristics in common with WGA, gives an indication in this direction.

In the present paper, we describe the purification and partial characterization of two lectins from rye (*Secale cereale*) and barley (*Hordeum vulgare*) embryos. Our results demonstrate that these lectins are almost identical with WGA, and thus represent two other members of the class of cereal lectins.

Abbreviation used: WGA, wheat-germ agglutinin.

### Materials and methods

#### Purification procedure

Since both rye and barley lectins are exclusively present in the embryos, isolated embryos were used as the material to start with. Large-scale isolation of rye embryos was done as described previously (Carlier & Peumans, 1976). For the isolation of barley embryos, which have a relatively large scutellum (unlike wheat and rye embryos), the pore size of the sieves had to be adapted. Moreover, barley embryo preparations are contaminated with husk fragments. This does, however, not affect the efficiency of the purification of the lectins. Embryos were soaked overnight in 10 vol. of acetate buffer (50 mM-sodium acetate, pH 4.5) and homogenized with a mixer. The homogenate was filtered through cheesecloth and centrifuged for 10 min at 10 000 *g*. The resulting supernatant was heated for 10 min at 60°C, cooled in an ice bath and cleared by centrifugation (10 min at 10 000 *g*). During this step a large amount of protein is removed without any loss of agglutination activity. Subsequently the cleared extracts were neutralized with 1 M-KOH, whereby another part of the proteins coagulated (especially in barley embryo extracts; for the isolation of rye lectins this step can be omitted). After removal of the coagulated material by centrifugation, the final supernatants were applied on to a column of immobilized *N*-acetylglucosamine (Selectin 1 from Pierce Chemical Company). Unbound protein was eluted with acetate buffer until the  $A_{280}$  fell below 0.01. Then water was applied and finally phosphate-buffered saline. With phosphate-buffered saline as an eluent, some protein (devoid of lectin activity) could be washed from the column. The bound lectins were desorbed with 0.1 M-*N*-acetyl-

glucosamine in phosphate-buffered saline and dialysed extensively against phosphate-buffered saline. If necessary the lectins can be concentrated by  $(\text{NH}_4)_2\text{SO}_4$  precipitation.

#### *Preparation of red blood cells*

Red blood cells were prepared from human or animal blood by repeated washing with phosphate-buffered saline. Trypsin-treated red blood cells were prepared by incubating a 10% suspension of washed red blood cells with 1mg of trypsin/ml for 1h at 37°C, followed by extensive washing with phosphate-buffered saline.

#### *Agglutination assays*

Agglutination assays were carried out in small glass tubes in a final volume of 0.1 ml containing 80  $\mu\text{l}$  of a 1% suspension of red blood cells (trypsin-treated or untreated) and 20  $\mu\text{l}$  of lectin solution or crude extract (each serially diluted with 2-fold increments). Agglutination was controlled visually after 1h.

#### *Ion-exchange chromatography*

Purified lectins were applied on to a column of SP-Sephadex (C 50) maintained in 50mM-sodium acetate buffer (pH 3.8) containing 0.1M-NaCl. The column was eluted with a linear gradient from 0.1 to 0.7 M-NaCl. Fractions (2 ml each) were collected and the  $A_{280}$  determined.

#### *Sodium dodecyl sulphate / polyacrylamide-gel electrophoresis*

This was done on 12.5–25% polyacrylamide gradient gels as described previously (Peumans *et al.*, 1980).

#### *Double immunodiffusion*

Double immunodiffusion was done in small Petri dishes (4 cm in diameter) filled with 2 ml of 1% agarose containing 4% poly(ethylene glycol) (mol.wt. 6000) and 0.1M-N-acetylglucosamine (to prevent aspecific binding of the lectin to immunoglobulins). After 24 h, unprecipitated proteins were eluted with phosphate-buffered saline, and the gels stained with 0.1% Coomassie Brilliant Blue in a mixture of acetic acid/methanol/water (1:5:5, by vol.). Thereafter, the gels were destained with the same solvent.

#### *Amino acid analysis*

Amino acid analyses of the purified lectins were done by Dr. B. Peeters (Laboratory for Human Biology, Faculty of Medicine, Katholieke Universiteit Leuven).

Lectin samples were hydrolysed (in sealed glass tubes under an  $\text{N}_2$  atmosphere) for 24 h at 110°C in 6 M-HCl containing 0.02%  $\beta$ -mercaptoethanol. After

hydrolysis, HCl was removed by evaporation, and the residue was dissolved in sodium citrate buffer (pH 1.9). Amino acid analysis was done with a Biotronik LC 2000 amino acid analyser. This apparatus allows a separation of all 20 amino acids on a single column of Durum-resin, using an elution programme consisting of four different buffers. Amino acids were directly quantified by using an Infotronics integrator. Tryptophan was determined after hydrolysis with 3 M-mercaptoethanesulphonic acid. Cysteine was determined as cysteic acid after hydrolysis in performic acid.

## **Results**

### *Yield*

The yield of rye and barley lectins is usually around 1mg per 10g embryos. About a quarter of the agglutinating activity of the initial extract is lost during the purification.

### *Agglutination properties and specificity*

Purified rye and barley lectins, as well as crude extracts from embryos, agglutinate red blood cells from human and animal origin. The agglutination is not blood-group-specific since the lectins agglutinate red blood cells of types A, B, AB and O equally well. Animal erythrocytes (from rabbit and pigeon) are agglutinated at the same lectin concentration as human red blood cells. Agglutination occurs at lectin concentrations as low as 0.5  $\mu\text{g}/\text{ml}$  and 0.02  $\mu\text{g}/\text{ml}$  with untreated and trypsin-treated red blood cells respectively. Crude extracts of rye and barley embryos were still active in agglutination assays at 64- and 1024-fold dilutions with untreated and trypsin-treated red blood cells respectively. The specificity of both lectins was determined with a series of simple sugars (glucose, galactose, lactose, raffinose, sucrose, mannose, fucose, maltose, melibiose, trehalose, arabinose, cellobiose, glucosamine, galactosamine, N-acetylglucosamine and N-acetylgalactosamine, all at final concentrations of 100mM). Only N-acetylglucosamine was found to inhibit the agglutination. The minimal concentration, at which N-acetylglucosamine inhibits the agglutination, was 20mM. Oligomers of N-acetylglucosamine (chitin hydrolysate) were much more effective inhibitors of the agglutination (at 100  $\mu\text{g}/\text{ml}$  the agglutination is completely inhibited).

### *Chemical and physical properties*

Both rye and barley lectins migrate as single polypeptide bands on sodium dodecyl sulphate/polyacrylamide gels with the same mobility as WGA (Fig. 1), indicating that their basic units have a mol.wt. around 18000. The native lectin molecules sediment into sucrose gradients with the same S-value as native WGA (3.6 S) and thus correspond

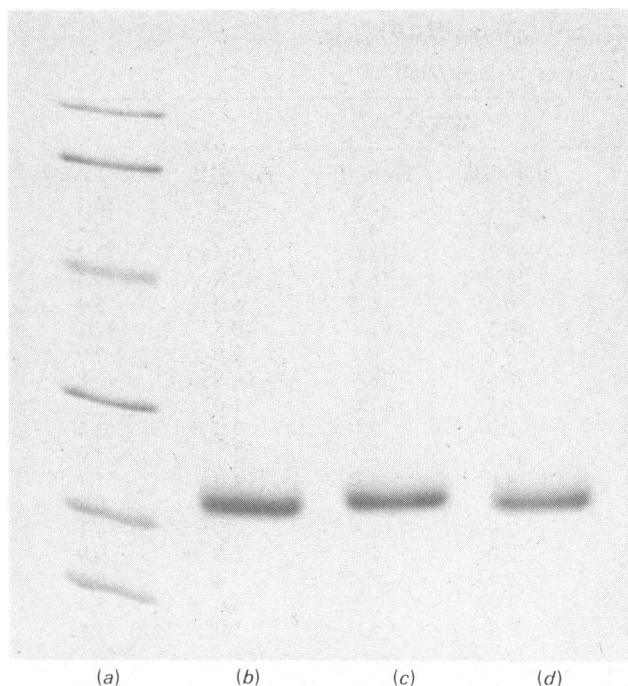


Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of rye and barley lectins and WGA on a 12.5–25% gradient gel (Peumans *et al.*, 1980)

Tracks (a), (b), (c) and (d) were loaded with marker proteins, WGA, rye lectin and barley lectin respectively. The molecular-weight-marker proteins are lysozyme (14 300), soya-bean trypsin inhibitor (21 000), carbonic anhydrase (30 000), ovalbumin (45 000), bovin serum albumin (67 000) and phosphorylase *b* (94 000).

to a protein with mol.wt. 36 000. It is obvious, therefore, that both lectins are dimers composed of two identical subunits. When centrifugation is carried out under conditions favouring dissociation of the lectin molecules (at a pH below 2.8), the sedimentation constant of the lectin is drastically reduced so that it corresponds to that of a protein with mol.wt. 18 000. As has been observed for WGA, rye and barley lectins have no sugar-binding activity when they occur in their dissociated form (so they can be eluted from the Selectin column by 0.1M-acetic acid). After enhancing the pH, the monomers reassociate to form fully active molecules. Ion-exchange chromatography of rye and barley lectins on sulphopropyl(SP)-Sephadex reveals single symmetrical peaks (Fig. 2), indicating that both lectins represent single molecular species. We have analysed also the elution pattern of the lectins from two other barley species, i.e. *Hordeum spontaneum* (which is considered as the direct ancestor of cultivated barley) and *Hordeum murinum*, which is a

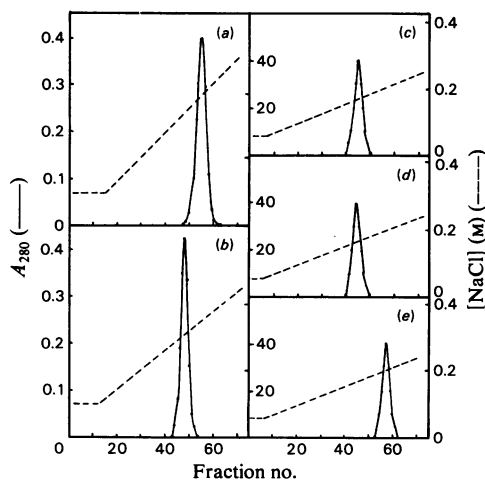


Fig. 2. Ion-exchange chromatography on sulphopropyl-Sephadex of rye (a) and barley (b) lectins and of lectins in crude extracts from embryos of *H. vulgare* (c), *H. spontaneum* (d) and *H. murinum* (e)

In (a) and (b) the lectins were applied on to a column (1 cm × 20 cm) of SP-Sephadex (C 50) maintained in 50 mM-sodium acetate, pH 3.8, containing 0.1 M-NaCl, and eluted with a linear gradient (200 ml) from 0.1 to 0.7 M-NaCl. Fractions (2 ml) were collected. The  $A_{280}$  was measured in a Beckman spectrophotometer. In (c)–(e), crude extracts from 100 mg embryos (in 2 ml of sodium acetate buffer, pH 3.8) were applied on to a column (1 cm × 10 cm) of SP-Sephadex (C 50) maintained in acetate buffer, and eluted with a linear gradient (100 ml) from 0.1 to 0.7 M-NaCl. Fractions (1 ml) were collected and assayed for agglutinating activity ( $A_{280}$ ) with trypsin-treated red blood cells. The agglutination titre is expressed as the highest dilution at which the fractions still agglutinated the red blood cells.

wild species. As shown in Fig. 2, the lectins from cultivated barley (*H. vulgare*) and *H. spontaneum* elute at exactly the same position. The lectin from *H. murinum*, however, eluates at a higher salt concentration. Amino acid analysis of rye and barley lectins (Table 1) shows that they are particularly rich in glycine and half-cystine residues.

#### Cross-reactivity with anti-WGA immunoglobulins

Rye lectin was challenged with a partially purified antiserum against WGA. As shown in Fig. 3, the precipitin lines formed with WGA and rye lectin fuse completely, which indicates that both lectins are antigenically closely related.

Double immunodiffusion of WGA and barley lectin against partially purified anti-WGA antiserum results in the formation of completely fused

Table 1. Amino acid composition of different cereal lectins

Amino acid	Content (residues/mol)						Rice lectin†
	Rye lectin	Barley lectin	WGA*				
			Form I	Form IIa	Form IIb	Form III	
Asx	16	15	14.9	15.1	15.4	15.1	16.1
Thr	4	5	5.0	4.0	4.1	4.0	2.8
Ser	15	12	13.7	13.5	13.0	13.5	13.2
Glx	18	17	16.4	16.0	16.6	15.8	17.3
Pro	12	9	5.2	6.2	6.3	6.0	5.4
Gly	40	40	41.0	40.5	41.1	40.5	37.1
Ala	8	10	10.1	9.4	9.4	9.8	7.9
½Cys	38	38	37.7	36.6	38.7	38.8	43.8
Val	1	2	0.9	1.1	1.0	1.0	—
Met	2	2	2.0	2.0	2.0	2.0	2.0
Ile	2	2	2.1	2.1	2.2	2.1	2.0
Leu	4	4	4.0	4.0	4.0	4.0	5.1
Tyr	8	8	8.0	7.4	8.0	7.6	8.8
Phe	1	2	3.3	2.9	2.4	2.9	4.1
His	2	1	0.0	2.0	1.8	2.0	0.9
Lys	8	9	6.8	7.4	7.8	7.4	6.6
Arg	3	3	4.0	3.4	3.5	4.0	5.4
Trp	1	1	3.2	2.9	3.3	3.0	1.8
Total	182	180	176	177	180	179	180.3
Calculated mol.wt.	18273	17946					

\* From Rice & Etzler (1975).

† From Tsuda (1979).

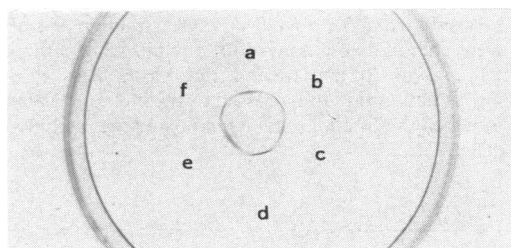


Fig. 3. Double immunodiffusion of rye and wheat lectin against anti-WGA immunoglobulins

Partially purified antiserum (against WGA) was applied in the central well. The peripheral wells were filled with 0.5 µg of rye (a, b and c) or wheat (d, e and f) lectin.

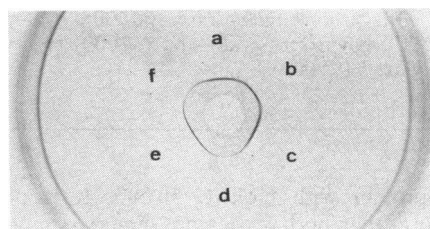


Fig. 4. Double immunodiffusion of barley lectins and WGA against anti-WGA immunoglobulins

Partially purified antiserum (against WGA) was applied in the central well. The peripheral wells were filled with WGA (a and e) and lectins from barley (b), *H. spontaneum* (c) and *H. murinum* (d and f).

precipitin lines. The lectins from *H. spontaneum* and *H. murinum* are also antigenically indistinguishable from each other and from WGA (Fig. 4).

### Discussion

Both rye and barley embryos contain a lectin with chemical, physical and biological properties almost

identical with those of WGA. They have the same molecular form, molecular weight, specificity, dissociation characteristics, amino acid composition and antigenic determinants as WGA (Nagata & Burger, 1974; Rice & Etzler, 1974; Rice & Etzler, 1975). It is obvious, therefore, that both wheat, rye and barley lectins resemble strongly each other, and thus belong to the same class of proteins, which can be designated as cereal lectins.

There is only one major difference between WGA and rye and barley lectins. The latter two behave as single molecular species, whereas WGA represents a mixture of three isolectins. Previous work on lectins from hexaploid and tetraploid wheats led to the conclusion that the multiple forms of WGA find their origin in the complex nature of the wheat genome. Allohexaploid wheat (genome formula AABBDD) and allotetraploid (AABB) wheat contain three and two isolectins respectively (Rice, 1976). Thus each individual genome directs the synthesis of its own lectin in these polyploid species. Since rye and barley are diploid species, their genomes are likely to contain the genes for one single lectin species.

The amino acid composition of rye and barley lectins and the three different isolectins of wheat (Rice & Etzler, 1975) show a high degree of similarity. Moreover, even the lectin from rice (Tsuda, 1979) had almost the same amino acid composition. We can conclude therefore that all the cereal lectins that have been analysed so far have the same unusual amino acid composition, characterized by extremely high levels of glycine and cysteine.

In addition to this, cereal lectins appear antigenically also to be related. The formation of completely fused precipitin lines with WGA and rye and barley lectins (Fig. 4) shows that these three proteins are in fact indistinguishable, and hence are very closely related to each other. All these observations provide evidence that during the evolution and divergency of the *Triticinae* sub-family of the *Gramineae*, the lectins of the species belonging to different genera have been highly conserved. Although such a high degree of conservation is not a direct proof for any function of the cereal lectins, it is a positive argument for a specific physiological role of this peculiar class of proteins.

Finally, it should be mentioned that the barley lectin that is described in the present paper does not

resemble the previously described agglutinating proteins from barley embryos (Foriers *et al.*, 1975, 1976) or whole-barley meal (Partridge *et al.*, 1976).

We thank Dr. B. Peeters (Department of Human Biology, Faculty of Medicine, Katholieke Universiteit Leuven) for amino acid analyses. The research was supported in part by grants from the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek. W. J. P. is Bevoegdverklaard Navorsers of this foundation.

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