Isolation and partial characterization of a lectin from a false brome grass (*Brachypodium sylvaticum*)

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(Received 21 May 1982/Accepted 22 June 1982)

A lectin has been isolated from embryos of a false brome grass species (*Brachypodium sylvaticum*) by affinity chromatography on immobilized *N*-acetylglucosamine. It is a dimeric protein of two identical subunits of mol.wt. 18 000. Although it resembles cereal lectins with respect to its biochemical and physicochemical properties, it differs structurally in several aspects from wheat-germ-agglutinin-like lectins.

Plant lectins have been intensively studied during the past decades by workers in diverse disciplines. As a result, the biochemical, physicochemical and biological properties of a steady increasing number of phytohaemagglutinins are well documented (Liener, 1976; Goldstein & Hayes, 1978). One of the best known examples is WGA, which has been characterized in detail, and, because of its peculiar biological properties, has become an important tool in cell-surface studies (Rice & Etzler, 1975; Wright, 1980). Until recently, WGA has been considered a unique lectin. However, the characterization of rye and barley embryo lectins revealed that these two lectins are almost identical with WGA (Peumans *et al.*, 1982a). In addition, further work on isolectins in wheats (Peumans *et al.*, 1982b) and subunit exchange between lectins from different cereal species (Peumans *et al.*, 1982c) strengthened the idea that cereal lectins represent a class of closely related proteins, which have been highly conserved during the evolution of the Triticeae tribe.

In the present paper, we describe the isolation and partial characterization of a lectin from the wild-grass species *Brachypodium sylvaticum* (false brome grass). Although this lectin resembles WGA, it differs in several aspects. In addition, an analysis of the immunological relationship between WGA and the *Brachypodium* lectin provides a chemotaxonomic tool, which can help to elucidate the controversial taxonomic classification of this genus.

Materials and methods

Materials

Seeds of *Brachypodium sylvaticum* were purchased by the Botanical Gardens of Leuven

Abbreviations used: BsyL, *Brachypodium sylvaticum* lectin; WGA, wheat-germ agglutinin.

(Belgium). Seed samples of *Brachypodium pinnatum* and *Brachypodium distachyon* were gifts from Dr. C. Lehmann (Zentral Institut für Genetik und Kulturpflanzenforschung der Akademie der Wissenschaften der DDR, Gatersleben, German Democratic Republic). Embryos were isolated from the seeds with a scalpel blade.

Lectin isolation

Embryos were homogenized in 10 vol. of sodium acetate buffer [50 mM-sodium acetate (pH 3.8)/0.1 M-NaCl] and the homogenate was centrifuged at 10 000 *g* for 4 min. The supernatant was taken off and applied to an affinity column of immobilized *N*-acetylglucosamine (Selectin 1; Pierce Chemical Company). Unbound protein was eluted with acetate buffer, water, phosphate-buffered saline and water in this sequence. Finally, the bound lectin was desorbed with 0.1 M-*N*-acetylglucosamine (in water).

Agglutination assays

Agglutination assays were carried out with trypsin-treated red blood cells as described previously (Peumans *et al.*, 1982a).

Ion-exchange chromatography

Purified lectin or crude embryo extracts were applied to a column (0.4 cm × 8 cm) of sulphopropyl-Sephadex (SP-Sephadex C-50) maintained in either 50 mM-sodium acetate, pH 3.8, containing 0.1 M-NaCl or 0.03 M-Tris/acetate (pH 5.0). Columns were eluted with a linear gradient from 0.1 to 0.7 M-NaCl (in sodium acetate buffer) or 0.03 to 0.3 M-Tris/acetate (pH 5.0) respectively. Fractions (0.5 ml) were collected and assayed for agglutination activity to determine the elution position of the lectins.

Subunit exchange experiments

Extracts were prepared from *Brachypodium sylvaticum* and *Triticum monococcum* embryos in 0.03 M-Tris/acetate (pH 5.0). Portions containing equal amounts of agglutinating activity were combined and dialysed against 0.1 M-acetate (whereby the respective lectins dissociate into monomers) overnight. Then the mixed lectin solution was cleared by centrifugation (5 min at 3000 g) and dialysed against 0.03 M-Tris/acetate (pH 5.0) for at least 12 h (whereby the lectin monomers re-associate into dimers). Finally, the mixture of lectins was separated on an SP-Sephadex column at pH 5.0. As a control, a similar experiment was carried out with *Triticum monococcum* and *Secale cereale* embryo extracts, in which, under the same conditions, subunit exchange is known to occur (Peumans *et al.*, 1982c).

Synthesis of lectin in vivo in germinating wheat embryos

About 200 embryos were incubated for 3 h in 0.4 ml of water containing 20 μ Ci of [35 S]cysteine (sp. radioactivity 1200 Ci/mmol). Then the embryos were exhaustively washed with ice-cold distilled water and homogenized in 2 ml of sodium acetate buffer. The lectin fraction was isolated as described above and analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and fluorography.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and fluorography

Lectin fractions were analysed on 12.5–25% acrylamide gradient gels by the principle described by Laemmli (1971). Labelled lectins were detected by fluorography. After fixing and staining, the gels were immersed for 30 min in a 1 M solution of sodium salicylate, dried and exposed to X-ray films (Kodak X-Omat R film).

Results

Occurrence of lectin in *Brachypodium sylvaticum*

Extracts from *Brachypodium sylvaticum* embryos agglutinate red blood cells. The agglutination is not blood-group-specific, as type A, B, AB and O red blood cells are agglutinated equally well. Trypsin-treated red blood cells are about 16 times more susceptible to agglutination than untreated erythrocytes. The specificity of the lectin 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 a final concentration of 100 mM). Only *N*-acetylglucosamine was found to inhibit completely the agglutination.

N-Acetylgalactosamine gave a partial inhibition. Oligomers of *N*-acetylglucosamine (chitin hydrolysate) were much more effective inhibitors, no agglutination occurring at 100 μ g of chitin hydrolysate/ml.

Molecular structure and physicochemical characteristics

Purified BsyL migrates as a single polypeptide band on sodium dodecyl sulphate/polyacrylamide gels with the same mobility as WGA subunits (Fig. 1), indicating that the basic subunit has a mol.wt. around 18 000. Sucrose-density-gradient analysis revealed that BsyL sediments with exactly the same *s*-value as WGA, and hence represents a protein with mol.wt. around 36 000. When centrifugation is carried out under dissociating conditions (in 0.1 M-acetic acid) BsyL sediments at a strongly reduced rate (2.1 S with WGA subunits taken as standards) and behaves as a molecule with mol.wt. around 18 000. These results show that BsyL is a dimer composed of two identical subunits of mol.wt. 18 000.

Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of BsyL

(a) Reference proteins; (b) WGA; (c) BsyL. The molecular-weight-marker proteins are lysozyme (mol.wt. 14 000), soya-bean trypsin inhibitor (mol.wt. 21 000), carbonic anhydrase (mol.wt. 30 000), ovalbumin (45 000), bovine serum albumin (67 000) and phosphorylase *b* (94 000).

Ion-exchange chromatography of BsyL on SP-Sephadex at pH 3.8 revealed a single lectin peak, indicating that it represents only one molecular species (Fig. 2).

Synthesis of BsyL in vivo

Recent investigations with wheat and rye embryos showed that the lectins in these species are synthesized as high-molecular-weight precursors, that are subsequently post-translationally processed by a sort of vectorial processing (Stinissen *et al.*, 1982). To find out whether BsyL is synthesized and processed in a similar way, BsyL synthesized *in vivo* (in 3 h-germinated embryos) was analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and fluorography. As shown in Fig. 3, most of radioactivity in the lectin fraction co-migrates with the lectin polypeptide. However, there is also a band of radioactivity visible at the position of a protein with mol.wt. 28 000. Since these results are exactly in agreement with those obtained with wheat and rye embryos, it can be concluded that BsyL is synthesized as a high-molecular-weight precursor and subsequently vectorially processed. Detailed studies of lectin processing in *Brachypodium* are problematical because of the small size of the embryos of these grasses and the practical problems inherent in this feature.

Cross-reactivity of Brachypodium lectins with anti-WGA immunoglobulins

Extracts from *Brachypodium sylvaticum*, *Brachypodium pinnatum* and *Brachypodium distachyon* were challenged with partially purified antiserum against WGA. As shown in Fig. 4, extracts from all three species cross-reacted with anti-WGA immunoglobulins. Their precipitin lines fuse completely, indicating that the lectins from these *Brachypodium* species are antigenically indistinguishable. The precipitin lines of wheat and

Fig. 2. Ion-exchange chromatography of BsyL on sulphopropyl-Sephadex at pH 3.8

Fig. 3. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and fluorography of BsyL synthesized *in vivo*

The position of molecular-weight-marker proteins and authentic BsyL (arrow) is indicated.

Brachypodium extracts, however, do not fuse, which means that WGA and BsyL are immunologically related, but not identical.

Failure of subunit exchange between Brachypodium and Triticum monococcum lectins

Wheat, rye and barley lectins are structurally so closely related that they can exchange subunits *in vitro* (and *in vivo*) (Peumans *et al.*, 1982b,c). Since WGA and BsyL resemble each other in many respects and, in addition, are immunologically related, an experiment was set up to find out whether these two lectins can exchange their subunits. No subunit exchange could be demonstrated between BsyL and *Triticum monococcum* lectin. Since, under the same conditions, *Triticum monococcum* and *Secale cereale* lectins form heterodimers *in vitro*, the

Fig. 4. Double immunodiffusion of extracts from common wheat (a), *Triticum monococcum* (b), *Brachypodium sylvaticum* (c), *Brachypodium pinnatum* (d) and *Brachypodium distachyon* (e) against anti-WGA.

failure of BsyL to do so is not due to the experimental conditions, but has to be ascribed to structural differences between BsyL and cereal lectins.

Discussion

Embryos from *Brachypodium sylvaticum* contain a lectin that resembles WGA and other cereal lectins. It has the same molecular weight and molecular structure as cereal lectins, it is synthesized as WGA and rye lectin and it is immunologically related to WGA. There are, however, some structural differences between BsyL and cereal lectins, since BsyL is only immunologically related to, and not immunologically identical with, WGA and no subunit exchange occurs between BsyL and a cereal lectin.

The systematic position of the genus *Brachypodium* in the grass family has been questioned on several occasions. Classifications based on morphological characteristics assigned this genus to the Poeae tribe (Engler, 1964), to a separate tribe (Brachypodieae) (Hubbard, 1968) or to the Brachypodiinae subtribe of the Triticeae tribe (which groups also the Triticinae and Hordeinae subtribes) (Hilu & Wright, 1982). The resemblance between BsyL and cereal lectins indicates that *Brachypodium* is related to the Triticeae. However, since there is much less difference between the lectins from

wheat and barley (belonging to the Triticinae and Hordeinae subtribes respectively) than between the lectins from *Brachypodium* and wheat or barley, it is evident that the genus *Brachypodium* can not be assigned to a Brachypodiinae subtribe of the Triticeae at the same hierarchic level as the Triticinae and Hordeinae subtribes. On the basis of the results reported in the present paper, *Brachypodium* should be placed in a separate tribe of the Brachypodieae.

Irrespective of the correct systematic position of the genus *Brachypodium*, it is clear that false brome grasses contain lectins that are related to WGA. Thus, WGA-like lectins have been highly conserved not only within the limited group of the Triticeae (Peumans *et al.*, 1982a), but also in less related species. Since such a conservation is characteristic for proteins that fulfil an important biological function, that is an argument in favour of a specific and important physiological function for grass lectins.

This research was supported in part by grants from the Belgian National Fonds voor Wetenschappelijk Onderzoek. W. J. P. is Bevoegdverklaard Navorsers of this Foundation. H. M. S. acknowledges the receipt of a fellowship from the Belgian Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw. We thank Dr. C. Lehmann for seed samples.

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