Isolation and partial characterization of a lectin from *Euphorbia heterophylla* seeds

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An *N*-acetylgalactosamine-specific lectin was isolated from *Euphorbia heterophylla* seeds by affinity chromatography on cross-linked arabinogalactan. It is a dimeric protein of two identical subunits of M_r 32 000, and differs structurally from all previously known Euphorbiaceae lectins. Its distribution over the seed is typical in that it is merely confined to the primary axes.

Plant lectins (phytohaemagglutinins) represent a heterogeneous class of proteins or glycoproteins with the unique ability of recognizing and binding specific sugars or sugar-containing (macro)molecules. They have been intensively studied during the past decades, and an ever-increasing number of them have been purified and characterized (for reviews see Liener, 1976; Goldstein & Hayes, 1978). During the last few years evidence has been accumulating that phytohaemagglutinins represent a number of classes of more or less closely related proteins, each class being characteristic for a particular group of plant species. Examples of such classes of lectins are (some of) the legume lectins, the Solanaceae lectins and the cereal lectins (Hankins *et al.*, 1979; Kilpatrick *et al.*, 1980; Peumans *et al.*, 1982). Other lectins, on the other hand, do not have any (known) related counterpart, and in some plant Families different types of lectins have been detected. The purpose of the present investigation was to isolate lectins from species belonging to the Euphorbiaceae Family and compare them with the lectins from *Ricinus communis* (castor bean) and *Hura crepitans*. Several species were checked for the presence of lectins, but only one of them, *Euphorbia heterophylla*, was positive. The lectin was isolated and partially characterized.

Materials and methods

Materials

Seeds of tropical Euphorbiaceae species were obtained from the Botanical Garden of the Faculty

Abbreviations used: EH agglutinin, *Euphorbia heterophylla* agglutinin; RC agglutinin, *Ricinus communis* agglutinin; WG agglutinin, wheat-germ agglutinin; HC agglutinin, *Hura crepitans* agglutinin.

of Science, University of Kisangani, Kisangani, Zaïre. Seeds of temperate species were collected locally.

Chemicals were obtained from commercial sources and were of the highest quality available.

Extraction of *Euphorbia heterophylla* seeds

Since seed coats of *E. heterophylla* release large amounts of slime on imbibition or homogenization in aqueous medium, they were manually removed before the extraction of the seeds. Decoated seeds were ground in a mortar and extracted with 10 ml of phosphate-buffered saline (0.15 M-NaCl/3 mM-KCl/10 mM-phosphate buffer, pH 7.2). The homogenate was centrifuged at 10 000 *g* for 10 min, and the supernatant was filtered on filter paper (Whatman 3MM) in order to remove particles floating on top of it.

Affinity chromatography on cross-linked arabinogalactan

The clean supernatant was applied to a column (5 ml bed volume) of cross-linked arabinogalactan (Pierce Chemical Co., Rockford, IL, U.S.A.) equilibrated with phosphate-buffered saline. Unbound protein was eluted with phosphate-buffered saline and water until the A_{280} fell to below 0.01. The lectin was desorbed with 0.1 M-lactose in phosphate-buffered saline (Fig. 1) and subsequently dialysed against phosphate-buffered saline. By this procedure virtually all the agglutination activity (>98%) present in the crude extract was recovered.

Preparation of trypsin-treated erythrocytes and agglutination assays

Trypsin-treated human erythrocytes were prepared as described previously (Peumans *et al.*, 1982). Agglutination titres of crude extracts and

purified lectin preparations were determined, in small glass tubes, in a final volume of 0.1 ml containing 80 μ l of a 1% suspension of trypsin-treated erythrocytes and 200 μ l of extract or lectin solution diluted serially with 2-fold increments. Agglutination was monitored visually after the tubes had stood for 1 h.

Results

Occurrence of lectins in Euphorbiaceae species

To test whether other Euphorbiaceae species (from both tropical and temperate regions) besides *Ricinus communis* and *Hura crepitans* contain lectins, seed from *Mercurialis perennis*, *Mercurialis annua*, *Euphorbia helioscopia*, *Euphorbia lathyris*, *Alchornea cordifolia*, *Tetrachidium reticulatus*, *Tetrachidium niruri*, *Cyathogyne viridis*, *Mallotus oppositifolius* and *Euphorbia heterophylla* were checked for the presence of lectins. Extracts were prepared in phosphate-buffered saline and assayed for agglutination activity with trypsin-treated erythrocytes. Only *Euphorbia heterophylla* seeds were found to yield agglutination-positive extracts.

Purification and properties of EH agglutinin

EH agglutinin was purified by affinity chromatography on cross-linked arabinogalactan. As shown in Fig. 1, all the agglutination activity present in the extract was bound to the column and could be eluted with 0.1 M-lactose. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of affinity-purified EH agglutinin showed that the preparations we obtained were essentially pure, since it yielded one single band of stained protein. The M_r of this polypeptide was determined, by reference to M_r -marker proteins, to be 32 000 (Fig. 2). Gel filtration of native lectin molecules on Sephadex G-100 indicated an M_r of about 65 000 (same M_r as haemoglobin) (Fig. 3), which implies that EH agglutinin is a dimer composed of identical subunits of M_r 32 000.

Agglutination properties

Purified EH agglutinin as well as crude seed extracts agglutinate erythrocytes from human and animal origin. The agglutination is not blood-group-specific, since human types A, B, AB and O are agglutinated equally well. Agglutination occurs at lectin concentrations as low as 1.2 μ g/ml and 0.005 μ g/ml with untreated and trypsin-treated erythrocytes respectively. Hence trypsin-treated erythrocytes are about 250-fold more sensitive than are untreated erythrocytes in agglutination assays with EH agglutinin.

Sugar specificity

The sugar specificity of EH agglutinin was determined with a series of simple sugars (glucose,

Fig. 1. Affinity chromatography of EH agglutinin on cross-linked arabinogalactan. Experimental details are indicated in the text. —, A_{280} ; ----, agglutination activity.

Fig. 2. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of EH agglutinin on 12.5–25% acrylamide gradient gels (Laemmli, 1971).

(a) RC agglutinin (subunits of M_r 31 000 and 34 000). (b) EH agglutinin. The positions of M_r -marker proteins are indicated by the arrows (lysozyme, M_r 14 000; soya-bean trypsin inhibitor, M_r 21 000; carbonic anhydrase, M_r 30 000; ovalbumin, M_r 45 000; bovine serum albumin, M_r 67 000; phosphorylase b, M_r 94 000).

Fig. 3. Gel filtration of EH agglutinin on Sephadex G-100

The column (27 cm × 6 cm) was equilibrated with phosphate-buffered saline and eluted with the same buffer at a flow rate of 5 ml/h. The elution positions of haemoglobin (Hb; M_r 65 000) and cytochrome *c* (Cyt. *c*; M_r 12 000) were determined by measuring A_{420} (-----). The positions of WG agglutinin (WGA; —) and EH agglutinin (EHA;) was traced by determining their agglutination activities in the presence of 0.1 M-lactose and 0.1 M-*N*-acetylglucosamine respectively. The positions of RC agglutinin (RCA) and HC agglutinin (HCA) were determined in a separate experiment with haemoglobin, cytochrome *c* and WG agglutinin as marker proteins.

galactose, lactose, raffinose, sucrose, maltose, melibiose, fucose, mannose, cellobiose, arabinose, trehalose, glucosamine, galactosamine, *N*-acetylglucosamine and *N*-acetylgalactosamine) all at a final concentration of 100 mM. As shown in Table 1, *N*-acetylgalactosamine was the best monosaccharide inhibitor tested, being respectively 6 and 16 times as potent as lactose and galactose. Melibiose and fucose were slightly inhibitory, whereas raffinose and galactosamine had an effect only at concentrations above 100 mM.

EH agglutinin does not inhibit cell-free protein synthesis

Several plant lectins are highly toxic and inhibit protein synthesis in cells or cell-free systems (Olsnes, 1978*a,b*; Gasperi-Campani *et al.*, 1978). To find out whether EH agglutinin also inhibits protein synthesis, its effect was investigated on [3 H]leucine incorporation in a wheat-embryo cell-free system under conditions optimized for translation of the endogenous templates (Peumans *et al.*, 1980). Since within the concentration range 2.5–100 μ g/ml (at which highest concentration toxic lectins completely inhibit protein synthesis; Barbieri *et al.*, 1979) no inhibition was observed, it may be concluded that EH agglutinin is not an inhibitor of protein synthesis.

Table 1. Carbohydrate specificity of EH agglutinin and HC agglutinin

Experimental details are indicated in the text.

Sugar	Concn. required for 50% inhibition (mM)	
	EH agglutinin	HC agglutinin*
<i>N</i> -Acetylgalactosamine	1.5	0.98
Lactose	9.37	No inhibition
Galactose	25	7.81
Melibiose	37.5	15.6
Fucose	50	15.6
Raffinose	>100	31
Galactosamine	>100	No inhibition

* Data from Falasca *et al.* (1980).

Distribution of EH agglutinin and RC agglutinin over different seed parts

Since EH agglutinin differs from RC agglutinin in several aspects it seemed worthwhile to compare its distribution in different seed parts with that of RC agglutinin. Extracts were prepared from primary axes and endosperm, and their agglutination activity was determined. As shown in Table 2, RC agglutinin is almost exclusively located in the endosperm, whereas EH agglutinin is present predominantly in the primary axis. The absolute amounts of EH agglutinin in primary axes and endosperm were estimated by comparing the agglutination activity of the extracts with that of an EH agglutinin solution of known concentration. They amounted to 115 ng and 11 ng per primary axis and endosperm respectively. Taking into consideration the total (phosphate-buffered-saline-soluble) protein content, EH agglutinin represents 0.2% and 0.003% of the total protein content in primary axis and endosperm respectively. It appears therefore that EH agglutinin is present in much smaller quantities than are RC agglutinin and legume lectins, which represent up to a few percent of the total protein content of their respective seeds.

Stability of EH agglutinin in crude extracts under different conditions

The stability of EH agglutinin (in crude extracts) was investigated under different conditions of pH, temperature and NaCl concentration. The lectin was found to be stable in the range pH 5–9 and to withstand heating up to 55°C. At NaCl concentrations above 2 M the lectin is irreversibly inactivated.

Discussion

Besides RC agglutinin, which is one of the best characterized phytohaemagglutinins (Goldstein &

Table 2. Comparison of the distribution of RC agglutinin and EH agglutinin over different seed parts
Experimental details are indicated in the text. One unit of lectin is defined as the amount of lectin that agglutinates 0.1 ml of a 1% suspension of trypsin-treated erythrocytes.

	Weight/organ		Total protein content/organ		Total lectin content/organ	
	(mg)	(%)	(μ g)	(%)	(munits)	(%)
<i>Ricinus communis</i>						
Primary axis	2.25	0.52	35	1.7	6.8	0.026
Cotyledons	15.4	3.53	1170	5.96	61.6	0.23
Endosperm	412	95.8	18 100	92.3	26 300	99.7
<i>Euphorbia heterophylla</i>						
Primary axis	0.48	9.8	45.6	12.1	23.0	91
Endosperm	4.4	90.2	422	87.8	2.2	9

Hayes, 1978), only two lectins have previously been isolated from species belonging to the (enormous) Euphorbiaceae Family. These two lectins were both isolated from seeds of *Hura crepitans*, a tropical species. One of them, named hurin, is a galactose-specific lectin composed of two (or four?) identical subunits of M_r 33 000 (McPherson & Hoover, 1979), whereas the other (referred to in the present paper as HC agglutinin) is a tetrameric *N*-acetyl-galactosamine-specific lectin composed of four identical subunits of M_r 30 000 (Falasca *et al.*, 1980). A comparison of EH agglutinin with the Euphorbiaceae lectins described above indicates several differences between them. Indeed, unlike the galactose-specific RC agglutinin, which is a tetramer composed of two different subunits, EH agglutinin is a dimer of two identical subunits and displays specificity for *N*-acetyl-galactosamine. In addition, EH agglutinin seems not to cross-react with anti-serum against RC agglutinin (results not shown). Although EH agglutinin resembles the *N*-acetyl-galactosamine-specific HC agglutinin from *Hura crepitans* in several aspects (both have a preference for *N*-acetyl-galactosamine over galactose, both are not blood-group-specific and both are composed of subunits of similar M_r), there are some obvious differences between these two lectins. First, EH agglutinin is a dimer, whereas HC agglutinin is a tetramer (cf. also Fig. 3), and, secondly, both lectins differ considerably with regard to factors affecting their sugar-binding specificity (e.g. EH agglutinin is inhibited by relatively low concentrations of lactose whereas HC agglutinin is not inhibited by this sugar) (Table 1). A comparison of EH agglutinin with the *N*-acetyl-galactosamine-specific lectins from legume species [*Dolichos biflorus*, *Glycine max* (soya bean), *Phaseolus lunatus*, *Sophora japonica*] (Goldstein & Hayes, 1978) reveals several differences. First, EH agglutinin is a dimer whereas the legume lectins are tetramers composed of four identical subunits; secondly, EH agglutinin is not blood-group-specific, whereas the lectins from *Dolichos biflorus*, *Glycine*

max and *Phaseolus lunatus* are group-A-specific; thirdly, EH agglutinin occurs in much smaller amounts than their legume counterparts, which represent up to a few percent of total seed protein (Liener, 1976). It is also worthwhile to mention here that EH agglutinin differs from ricin, since this toxin is a dimer of two different subunits and, unlike EH agglutinin, is an extremely potent inhibitor of protein synthesis (Olsnes, 1978b). Besides the structural differences between RC agglutinin and EH agglutinin, these two lectins have also a completely different distribution over the different seed parts. RC agglutinin is almost exclusively localized in the storage tissue, namely in the endosperm, in which it occurs in protein bodies (Youle & Huang, 1976; Tully & Beevers, 1976), whereas EH agglutinin is mainly confined to the primary axis. These different localizations may be important in view of the possible physiological role(s) of Euphorbiaceae lectins. Summarizing, it can be concluded that the Euphorbiaceae lectins that have been isolated up to now show little structural similarities. However, since these lectins have been purified from seeds of species belonging to different genera, further exploration of this enormous Family will be required to find out whether other species contain lectins, and, if so, whether they are related to each other.

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