An unusual lectin from stinging nettle (*Urtica dioica*) rhizomes

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An unusual lectin has been isolated from stinging nettle (*Urtica dioica* L.) rhizomes. It is a small (8.5 kDa) monomeric protein with high contents of glycine, cysteine and tryptophan. The *U. dioica* agglutinin (UDA) is not blood group-specific and is specifically inhibited by *N*-acetylglucosamine oligomers. As compared to other plant lectins, UDA has a very low specific agglutination activity. Nevertheless, it induces HuIFN-γ in human lymphocytes at concentrations comparable to those of other inducers.

Urtica dioica *Lectin* *Interferon* *Lymphocyte*

1. INTRODUCTION

Plant lectins are a heterogeneous group of (glyco)proteins having in common the unique ability to recognize and bind specific sugar residues [1,2]. At present, over 100 plant lectins have been isolated and partially characterized with respect to their structures and specificities. Although we have the most information about legume seed lectins, it is becoming increasingly apparent that plants of other taxonomic groups also contain lectins, and not only in seeds but also in different types of vegetative tissues [1,2]. Moreover, evidence is accumulating that several plant lectins occur exclusively in vegetative tissues (e.g., roots, leaves, rhizomes, bark, fruits and phloem sap [3–8]). This report describes the isolation and partial characterization of an unusual agglutinin from rhizomes of stinging nettle (*Urtica dioica*), a typical representative of the family of the Urticaceae.

Abbreviations: UDA, *Urtica dioica* agglutinin; SDS–PAGE, SDS-polyacrylamide gel electrophoresis; Con A, concanavalin A; SEA, staphylococcal enterotoxin A

2. MATERIALS AND METHODS

2.1. *Material*

Underground rhizomes were collected during the winter period. After thorough washing with tap water, they were cut into small pieces (about 1 cm) and used immediately or stored at −20°C.

2.2. *Isolation of the* Urtica dioica *agglutinin*

Rhizomes (1 kg) were homogenized with a mixer in 5 vols of 0.1 N HCl. The homogenate was squeezed through a double layer of cheesecloth and centrifuged (10 min, 20000 × *g*). Then the resulting supernatant was brought to pH 3.8 (with 2 N NaOH), allowed to stand in ice for 1 h and cleared by centrifugation (10 min, 20000 × *g*). Finally, the cleared extract was filtrated through filter paper (Whatman 3 MM) to remove any particulate material left, and applied on a column of chitin (100 ml bed volume) equilibrated with acetate buffer (50 mM Na acetate, pH 3.8, containing 0.1 M NaCl). Before use, the chitin (practical grade powder from Sigma, St. Louis, MO) was extensively washed with 1 N HCl, H_2O, 1 N NaOH, 1 M NaCl in H_2O and acetate buffer successively. After passing the extract, unbound pro-

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tein was washed off with 1 M NaCl in acetate buffer until the A_{280} fell below 0.01 and the lectin desorbed with 0.5 N acetic acid. Fractions containing agglutination activity were collected and brought at 60% $(NH_4)_2SO_4$ (relative saturation). Precipitated proteins were collected by centrifugation (20 min, 20000 × *g*), redissolved in acetate buffer and rechromatographed on chitin. Again, the lectin containing fractions were collected and the agglutinin precipitated with $(NH_4)_2SO_4$. After centrifugation (20 min, 20000 × *g*), the lectin pellet was dissolved in acetate buffer (250 ml) and applied on a column (2.6 × 40 cm, 150 ml bed volume) of sulfopropyl-Sephadex (SP-Sephadex, type C 50, Pharmacia) preequilibrated with acetate buffer. Unbound protein was eluted by washing the column with 100 ml acetate buffer, prior to desorbing the lectin with a linear gradient (750 ml) of NaCl (0.1–0.5 M) in acetate buffer. Peak fractions were pooled and the lectin precipitated with $(NH_4)_2SO_4$. Finally, the lectin pellet was dissolved either in H_2O or an appropriate buffer solution.

2.3. *Assays and analysis methods*

Protein was determined as in [9]. Total neutral sugar was estimated using the phenol-sulfuric acid method [10]. Agglutination assays were carried out with trypsin-treated or untreated human erythrocytes as in [5]. SDS–PAGE was done on 12.5–25% polyacrylamide gradient gels using a discontinuous system as in [11]. Fluorography was performed with 'Amplify' (Amersham) as a scintillator. Amino acid analysis was carried out as in [6].

2.4. *Induction of interferon in fresh human lymphocytes*

Peripheral blood lymphocytes were isolated from a total buffy coat of a healthy donor by Ficoll-sodium Metrizoate (Lymphoprep, Nyegaard, Oslo) centrifugation [12]. After washing the lymphocytes twice with Dulbecco's phosphate-buffered saline, they were resuspended at a density of 2×10^6 cells per ml in medium RPMI 1640 (Gibco, Paisley, Scotland), supplemented with 10% (v/v) autologous plasma. Two-ml cell cultures were seeded in 24-well semi-microtiter plates and different amounts of the lectin added. Cultures induced with Con A and SEA were included as positive controls. After 3 days incubation at 37°C the supernatant of the cultures was removed, clarified (3000 rpm, 10 min) and titrated for antiviral activity on HEp-2 cells [13].

2.5. *Preparation of chitin hydrolysate*

A partially purified chitin hydrolysate was prepared as in [14] and tested with several lectins specific for *N*-acetylglucosamine oligomers. Lectins from rice embryos, wheat germ, potato tuber and Jimson weed seeds were inhibited (50%) at chitin hydrolysate concentrations of 10, 20, 25 and 100 μg/ml, respectively. Lectins with other specificities were not inhibited at all.

3. RESULTS

3.1. *Occurrence, agglutination properties and carbohydrate-binding specificity of the lectin in extracts from stinging nettle rhizomes*

Crude extracts from rhizomes of stinging nettle exhibit a (low) agglutination activity, at least when tested with trypsin-treated red blood cells. In other parts of the plants (e.g., leaves, stems, flowers and seed) no lectin activity could be detected in crude extracts. Since erythrocytes of group A, B, AB and O were agglutinated equally well, the lectin is apparently not blood group-specific. The sugar specificity of the lectin in crude extracts was determined with a series of simple sugars (*N*-acetylgalactosamine, lactose, melibiose, fucose, galactose, galactosamine, raffinose, stachyose, glucose, sucrose, mannose, trehalose, arabinose, cellobiose, glucosamine, *N*-acetylglucosamine, ribose) all at a final concentration of 100 mM, and a mixture of *N*-acetylglucosamine oligomers (20 μg/ml of a partially purified chitin hydrolysate). Only *N*-acetylglucosamine oligomers were found to be inhibitory.

Since the stinging nettle lectin was specific for *N*-acetylglucosamine oligomers, it was purified by affinity chromatography on chitin. When isolated as described in the methods section, the yield was about 1 g pure UDA per kg rhizomes. Extraction of rhizomes has to be done at low pH (e.g., in 0.1 N HCl or 0.5 N acetic acid) since extracts prepared in a neutral buffer solution (e.g., phosphate-buffered saline) exhibit a 10-fold lower agglutination activity.

3.2. *Molecular structure of DNA*

Purified UDA was analyzed by SDS–PAGE with and without reduction with 2% β-mercaptoethanol. In both cases, the lectin migrated with an apparent M_r of about 9000 (fig.1). The M_r of native UDA as estimated by gel filtration on Sephadex G-50 was about 3000 (fig.2a). However, since most lectins which are specific for *N*-acetylglucosamine oligomers are retarded on Sephadex gels, this method probably yields an underestimation of the M_r. Sucrose density gradient centrifugation of UDA indicated a M_r clearly lower than that of cytochrome *c* (M_r 12300) and wheat germ agglutinin (WGA) subunits (M_r 18000) (fig.2b). Moreover, the sedimentation velocity of UDA was the same both at neutral (7.4) and acid (2.8) pH, indicating that UDA does not dissociate into subunits in 0.5 N acetic acid (as do WGA and other cereal lectins). Based on these results, it can be concluded that native UDA behaves as a single polypeptide of M_r ~8000–9000.

Fig.1. SDS–PAGE of UDA in the presence (lane 3) and absence (lane 4) of mercaptoethanol. Lane 1 contains M_r marker proteins (lysozyme, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin, phosphorylase *b*). Lane 2 contains rice lectin polypeptides (with amino acid composition similar to that of UDA). M_r values of these polypeptides are 8000, 10000 and 18000 [15].

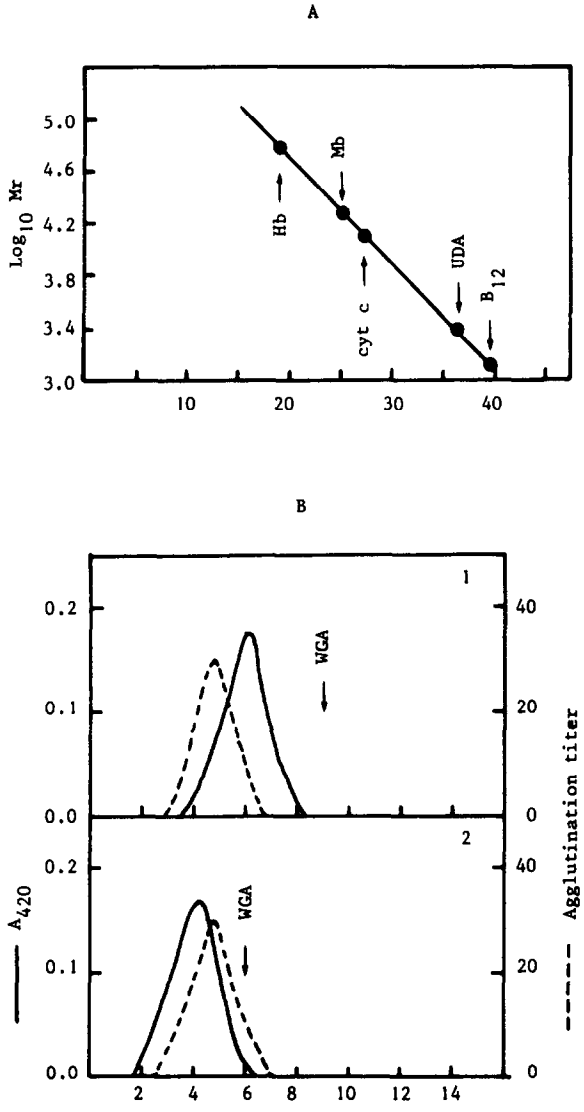

Fig.2. (A) Gel filtration of UDA on a Sephadex G-50 column (1.4 × 28 cm; phosphate-buffered saline as running buffer). Marker proteins were hemoglobin (Hb, M_r 65000), myoglobin (Mb, M_r 18000), cytochrome *c* (cyt c, M_r 12400) and vit.B_{12} (B_{12}, M_r 1300). (B) Sucrose density gradient centrifugation of UDA. Purified UDA was centrifuged in a 12–38% sucrose gradient [either in phosphate-buffered saline (panel 1) or 0.5 N acetic acid (panel 2)] at 2°C for 20 h in a Beckman SW 50.1 rotor. Gradients were fractionated in 0.2-ml portions and assayed for agglutination activity and A_{240} (to trace the position of cytochrome *c*). The position of WGA (run in parallel gradients) is indicated by the arrows. WGA (3.6 S in phosphate-buffered saline) dissociates into subunits (2.1 S) in 0.5 N acetic acid.

A more precise figure could be deduced from the amino acid analysis of UDA. An M_r of 8526 was calculated from the total number of amino acid residues. Since no carbohydrate was detected in purified UDA, covalently bound sugars need not be taken into account for the calculation of its M_r. As shown in table 1, amino acid analysis of UDA revealed that this protein is particularly rich in glycine and cysteine (18.2 and 15.6%, respectively). In addition, it has an unusually high content of tryptophan (9.1%) but lacks proline, methionine and phenylalanine.

Purified UDA is a very stable protein. It is not denatured by acid (0.1 N HCl, 1 N acetic acid or 5% trichloroacetic acid). Above pH 12, however, its activity is gradually lost. UDA withstands heating at 80°C for 15 min (at least in acetate buffer). Even after heating in a boiling water bath for 15 min, 50% of the initial agglutination activity was retained.

3.3. *Agglutination properties*

As could be expected from experiments with crude extracts, purified UDA exhibited specificity towards oligomers of *N*-acetylglucosamine. The inhibitory effect of chitin hydrolysate was highly specific as UDA was completely inhibited by the hydrolysate at a concentration of 1 μg/ml (which is 10–100-fold lower than that required for other lectins with similar sugar-binding specificity; cf. section 2.5). Moreover, the agglutination of erythrocytes by UDA could be fully reversed by the chitin hydrolysate. UDA exhibits a rather low specific agglutination activity when assayed with human erythrocytes. Indeed, the minimal concentration required for agglutination of trypsin-treated red blood cells was about 15 μg/ml, whereas most of plant lectins agglutinate the same erythrocytes at concentrations varying between 0.01 and 0.5 μg/ml. Untreated human red blood cells were poorly agglutinated by UDA, even at lectin concentrations as high as 2 mg/ml. However, when rabbit erythrocytes were used, UDA agglutinated trypsin-treated and untreated cells at concentrations as low as 2.5 and 25 μg/ml, respectively.

Table 1

Amino acid composition of UDA

Amino acid	mol%	Residues/mol
AsX	9.3	7
Thr	2.4	2
Ser	11.7	9
GlX	7.9	6
Pro	0.0	0
Gly	18.4	14
Cys	16.2	12
Ala	3.7	3
Val	2.7	2
Met	0.0	0
Ile	1.2	1
Leu	1.2	1
Tyr	3.6	3
Phe	0.0	0
His	2.2	2
Lys	2.1	2
Arg	7.8	6
Trp	9.7	7

Table 2

Induction of HuIFN-γ in human lymphocytes by UDA and some other inducers

Inducer	Antiviral activity (log_{10} IU/ml)
None	<1.3
Con A, 10 μg/ml	3.1
SEA, 1/1000	2.5
UDA, 3 μg/ml	<1.3
10 μg/ml	1.8
30 μg/ml	2.6
100 μg/ml	2.8
300 μg/ml	2.4

3.4. *Induction of interferon in human lymphocytes*

To determine whether UDA can interact with cells in a more specific way, its effect was investigated on the induction of interferon in human lymphocytes. As shown in table 2, UDA induced considerable amounts of antiviral activity in the cell cultures. Moreover, the antiviral activity produced after stimulating lymphocytes with UDA was characterized by using specific antisera as being due solely to HuIFN-γ (formerly called immune interferon).

4. DISCUSSION

Stinging nettle rhizomes contain considerable amounts of a lectin which exhibits carbohydrate-binding specificity for *N*-acetylglucosamine oligomers. UDA could be purified to homogeneity using a combination of affinity chromatography on chitin and ion-exchange chromatography on SP-Sephadex. Native UDA behaves as a monomeric protein composed of a polypeptide chain of 77 amino acids with no covalently bound carbohydrate. Its amino acid composition is typified by high contents of glycine and cysteine and the presence of more than 9% tryptophan. Compared to other plant lectins, UDA has an extremely low specific agglutination activity. Nevertheless, UDA has to be considered as a lectin since its agglutination activity can be inhibited by *N*-acetylglucosamine oligomers. Moreover, UDA also interacts with cells in a highly specific manner as illustrated by the (concentration-dependent) induction of interferon in lymphocytes. UDA is the first lectin to be isolated from a member of the Urticaceae family and differs definitely from all other known plant lectins with respect to its molecular structure. It is not only the smallest phytohemagglutinin known at present, but also the first single-chain lectin to be found in plants.

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