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RELATED GLYCOPROTEIN LECTINS FROM ROOT STOCKS OF WILD CUCUMBERS

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Key Word Index—*Bryonia dioica*; *Marah macrocarpus*; Cucurbitaceae; white bryony; wild cucumber; root stock; characterization; glycoprotein; lectin.

Abstract—The lectin from root stocks of the Californian wild cucumber (*Marah macrocarpus*) has a molecular structure similar to that of the root stock lectin from white bryony (*Bryonia dioica*). These two Cucurbitaceae lectins (M_r around 60–65 000) are composed of two different subunits which are held together by disulphide bridges. They resemble each other in amino acid and carbohydrate composition and are serologically related. It appears that, during the evolution of the Cucurbitaceae family, the genes encoding root stock lectins of at least some perennial species have been conserved fairly well.

INTRODUCTION

During the last decade evidence has accumulated that plant lectins occur in different types of vegetative storage tissues of a number of species belonging to different taxonomic groups. Well-known examples of such phytohaemagglutinins are those found in potato tuber [1], pokeweed root [2], black locust [3] and elder [4] bark, and ground elder [5] and stinging nettle [6] rhizomes. In addition, root tuber lectins were isolated from two different Cucurbitaceae species, *Trichosanthes kirilowii* [7] and *Bryonia dioica* [8].

The present report deals with the isolation and characterization of a glycoprotein lectin from a root stock of the wild cucumber *Marah macrocarpus* (Cucurbitaceae) which is indigenous to California, U.S.A.

RESULTS AND DISCUSSION

Molecular structure of Bryonia dioica *agglutinin (BDA) and* Marah macrocarpus *agglutinin (MMA)*

Purified lectins were analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) both in the presence and absence of mercaptoethanol. As shown in Fig. 1, unreduced BDA and MMA migrate as single bands of M_r 61 000 and 64 000, respectively. After reduction with mercaptoethanol, however, BDA as well as MMA yielded two bands with M_r 32 000 and 30 000, and 34 000 and 31 500, respectively. The M_r of native BDA and MMA was estimated by gel filtration and sucrose-density gradient centrifugation. Both methods (results not shown) indicated a M_r *ca* 60–65 000 for both lectins. It appears, therefore, that BDA as well as MMA are composed of two different subunits held together by disulphide bridges.

Amino acid and carbohydrate composition

The amino acid and carbohydrate composition of BDA and MMA are given in Table 1. The lectins show some resemblance to each other in amino acid and carbohydrate content with high levels of aspartic and glutamic acids, serine and leucine. In addition, the lectins have a similar sugar composition with glucosamine (presumably *N*-acetylated), mannose, fucose, xylose and glucose being present. The MMA, however, has about three times as much carbohydrate. Most probably, this higher carbohydrate content explains the higher M_r of MMA as compared to that of BDA.

Comparison of the agglutination properties and carbohydrate specificities

Both BDA and MMA agglutinate human type A, B and O and rabbit erythrocytes almost equally well (Table 2). Trypsin-treated human red blood cells are about 10 and 20 times better agglutinated than untreated cells by BDA and MMA, respectively, whereas trypsinization of rabbit erythrocytes increases their sensitivity to both lectins only by about 5-fold. The main difference between BDA and MMA with respect to their agglutination properties is that the former has a considerably higher specific agglutination activity. From the inhibition studies with the mono- and oligosaccharides it can be concluded that BDA and MMA exhibit a different specificity. Indeed, although both lectins are best inhibited by *N*-acetylgalactosamine, MMA is much better inhibited by fucose, galactose and raffinose than BDA (Table 3).

Serological relationships between BDA and MMA

To determine possible serological relationships between BDA and MMA, an antiserum was prepared

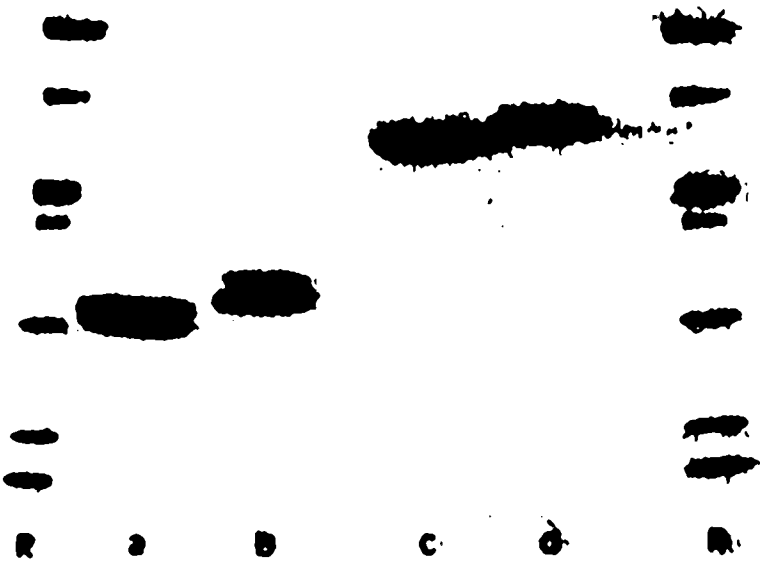

Fig. 1. SDS-PAGE of BDA and MMA on 12.5–25% acrylamide gradient gels [11] in the presence (a, b) and absence (c, d) of 2% 2-mercaptoethanol. BDA and MMA were loaded on slots a and c, and b and d, respectively. M_r reference proteins (lanes R) were lysozyme (M_r 14 300), soyabean trypsin inhibitor (M_r 21 000), carbonic anhydrase (M_r 30 000), ovalbumin (M_r 45 000), bovine serum albumin (M_r 67 000) and phosphorylase *b* (M_r 94 000). Gels were stained with Coomassie brilliant blue.

against BDA and challenged with purified BDA and MMA in double immunodiffusion assays. As shown in Fig. 2, MMA clearly cross-reacted with anti-BDA indicating that both lectins are serologically related.

As has been reported previously, the two wild perennial Cucurbitaceae species *T. kirilowii* and *B. dioica* contain lectins in their root stocks [7, 8]. A comparison of both lectins indicates that they have a similar molecular structure as they are built up of two different subunits of M_r around 30–35 000. However, whereas BDA is a *N*-acetylgalactosamine-specific agglutinin, the *T. kirilowii* lectin exhibits specificity towards galactose. In addition, the latter lectin appeared to be a mixture of three isolectins with different M_r unlike BDA which consists of a single molecular species. A more detailed comparison of BDA to the root stock lectin from the Californian wild cucumber (*Marah macrocarpus*), which is described here, indicates a striking similarity between both lectins isolated from two different indigenous species from two different continents. Indeed, BDA and MMA not only have the same basic molecular structure but also similar amino acid and carbohydrate compositions. Moreover, structural similarities between both lectins are further inferred from the cross-reactivity of MMA with anti-BDA. It appears, therefore, that during the evolution of the Cucurbitaceae family, the genes encoding lectins in root stocks of at least some perennial species have been conserved fairly well, even when considering that different taxons have evolved quite independently on different continents. However, in spite of the obvious structural similarities between both glycoprotein lectins, their agglutination properties and sugar binding specificities are definitely different, which as in the case of legume lectins, indicates that the overall molecular structure has been conserved better than the sugar binding sites.

EXPERIMENTAL

Materials. Root stocks of *Bryonia dioica* and *Marah macrocarpus* were collected locally in the vicinity of Leuven (Belgium) and La Jolla, CA (U.S.A.), respectively.

Isolation of lectins from B. dioica *and* M. macrocarpus *root stocks.* Both the *B. dioica* agglutinin (BDA) and *M. macrocarpus* agglutinin (MMA) were isolated by affinity chromatography on fetuin-agarose essentially as described previously [8]. After the affinity chromatography step, both lectins were further purified by ion-exchange chromatography using a Pharmacia Fast Protein Liquid Chromatography system (type GP 250). Fractions pooled after affinity chromatography were dialysed overnight against 20 mM 1,3-diaminopropane (DAP)–HCl (pH 9) buffer and applied to a column of anion exchanger of Mono Q type HR 5/5 (Pharmacia) equilibrated with the same buffer. After the column had been washed with 20 mM DAP (pH 9), elution was performed using a linear salt gradient (0–0.3 M NaCl) in 20 mM DAP, pH 9. Both BDA and MMA yielded single symmetrical peaks eluting at 0.15 and 0.2 M NaCl, respectively (results not shown).

Haemagglutination assays were carried out in small glass tubes in which 80 μl of a 1% suspension of human trypsin-treated erythrocytes [9] were added to 20 μl portions of crude extract or purified lectin solution double serially diluted. Agglutination was then assessed by eye after the tubes had been left for 1 hr at room temp.

Table 1. Amino acid and sugar composition of MMA and BDA

Amino acid	MMA (mol %)	BDA* (mol %)
Asx	11.9	14.3
Thr	5.1	6.4
Ser	16.9	10.5
Glx	11.7	9.3
Pro	5.1	3.8
Gly	11.3	6.1
Ala	7.7	6.1
1/2 Cys	1.8	N.D.
Val	4.7	7.1
Met	0.2	1.4
Ile	3.7	5.2
Leu	6.0	8.4
Tyr	2.7	4.0
Phe	2.7	4.3
His	2.0	1.1
Lys	3.2	6.0
Trp	N.D.	1.7
Arg	3.3	4.3
Sugar		
Glucosamine	1.61	0.50
Fucose	0.51	0.07
Xylose	0.60	0.14
Mannose	2.68	0.74
Galactose	0.58	0.27
Glucose	1.02	0.43
% (w/w)	10.7	3.3

*Data for amino acids from [8] with corrections.

N.D., not determined.

Table 2. Agglutination activity of BDA and MMA with different types of red blood cells

Red blood cells*	Agglutination titre BDA (0.1 mg/ml)	MMA (0.1 mg/ml)
A_{UT}	120	10
A_{TT}	1280	240
B_{UT}	120	10
B_{TT}	1280	240
O_{UT}	120	10
O_{TT}	1280	240
R_{UT}	240	40
R_{TT}	1280	240

*UT, untreated; TT, trypsin-treated.

Analyses. The amino acid and amino sugar composition was determined after hydrolysis in 3 M toluene-*p*-sulphonic acid on an amino acid analyser and the neutral sugar content after methanolysis and trimethylsilylation by GC [10].

Determination of molecular size by gel filtration. Purified BDA and MMA were analysed by gel filtration on a Sephadex G-100 column (1.4 × 28 cm; PBS as running buffer). M_r marker proteins were aldolase (M_r 160000), bovine serum albumin (M_r 67000), ovalbumin (M_r 45000), chymotrypsin (M_r 25000) and cytochrome *c* (M_r 13000). Their elution position was determined in a separate run.

Sucrose density-gradient centrifugation. BDA and MMA were centrifuged in a 12–38 % sucrose gradient (in PBS) at 2° for 24 hr at 50000 rpm in a Beckman SW 50.1 rotor. Gradients were fractionated with an ISCO density gradient fractionator. The sedimentation position of wheat germ agglutinin (WGA: 36000), human haemoglobin (Hb: 65000) and *Ricinus communis* agglutinin (RCA: 130000) was determined in a separate gradient.

Table 3. Carbohydrate binding specificity of BDA and MMA

Sugar	Minimal concentration* (mM) required for 50% inhibition with BDA (0.1 mg/ml)	MMA (0.1 mg/ml)
N-Acetylgalactosamine	0.8	0.8
Lactose	3.2	0.8
Melibiose	6.25	3.2
Fucose	12.5	1.6
Galactose	12.5	1.6
Galactosamine	2.5	12.5
Raffinose	50	6.25
Stachyose	100	12.5

*Determined with trypsin-treated human type A erythrocytes.

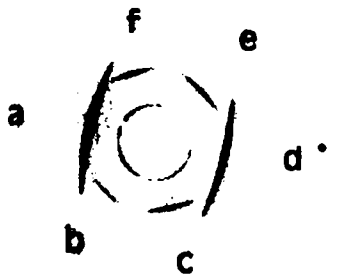

Fig. 2. Double immunodiffusion of BDA and MMA against BDA-antiserum. Five micrograms of purified BDA (a, d) and MMA (b, c, e, f) were challenged with BDA-antiserum (central well). After formation of precipitin lines, unprecipitated serum proteins were eluted by placing the gels overnight in PBS. Then, they were fixed and stained with Coomassie brilliant blue just as SDS-polyacrylamide gels.

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