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Communication

Salt-Soluble Lectins of Corn Grain

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ABSTRACT

Lectins extracted from corn (*Zea mays* L.) kernel with Tris-HCl buffer pH 7.5 were isolated from the crude extract by affinity chromatography on Sepharose 6B-*N*-acetyl-D-galactosamine and Sepharose 6B-methylα-D-mannoside, and also by lectin affinity chromatography using concanavalin A and *Lens culinaris* lectin as ligands. According to preferential monosaccharide specificity, salt-soluble lectins of corn seed comprise at least two distinct types: *N*-acetyl-D-galactosamine-interactive and mannose-interactive lectins. The extracted lectins are unstable, with a tendency to form aggregates during storage.

The study of Stinissen and coworkers in 1983 (11) was among the first to indicate that corn kernel contained a lectin with weak hemagglutinating activity. Using a modified hemagglutination assay, Newburg and Concon (9) demonstrated that corn endosperm contained not one but several lectins distributed between main extracted protein fractions, namely globulins, zein, and glutelins. These first results suggested that corn lectins differ from other lectins of Graminae family by their versatility, localization, and specificity.

Recently, Lepekhin and Ribak (8) isolated several lectins from corn germ and more directly demonstrated their distinctions from other cereal lectins and considerable intraspecies heterogeneity. However, despite evidence that corn seed contains lectins with peculiar characteristics, efforts to isolate and examine properties and physiological functions of these lectins are scarce. As a consequence, contemporary reviews on plant lectins completely omit this well examined species (3–5).

This paper presents the initial results of an attempt to isolate and discriminate corn kernel lectins belonging to the albumin-globulin protein fraction and to examine some of their properties.

MATERIALS AND METHODS

Material

Corn (*Zea mays* L.) was provided by Corn Research Institute, Zemun Polje, Yugoslavia. It was a commercial double-cross hybrid (ZP-SK-704) grown in 1987. Lectins applied for lectin affinity chromatography (Con A, LCA[1]) were isolated and purified by using appropriate procedures (1, 10) and covalently bound to Sepharose 4B (Pharmacia, Sweden) by CNBr method. Monosaccharides (ManαOMe, Man, GalNAc, Gal) were purchased from Sigma and bound to epoxy-activated Sepharose 6B, as described by Vretblad (13). All other chemicals were reagent grade.

Extraction

Whole corn kernels (100 g) were ground in a hammer mill to pass a 40-mesh screen. The obtained meal was preextracted with 300 mL acetone for 2 h, washed with additional acetone in a Büchner funnel, and air-dried. The combined albumin-globulin fraction was extracted by stirring the defatted meal in 50 mM Tris-HCl buffer (pH 7.5) (5 mL buffer/g) overnight. Slurry was centrifuged at 3000 rpm for 30 min, and the supernatant was collected and adjusted to pH 5.0 by adding 0.1 M HCl. Some protein material precipitated after acidification and it was discarded after centrifugation. Clear supernatant was dialyzed overnight against the extraction buffer. It was used as starting material for separation of corn lectins of the albumin-globulin protein fraction. All these and subsequent operations were performed at 4°C.

Biospecific Affinity Chromatography

Affinity columns of Sepharose 6B-Gal, Sepharose 6B-GalNAc, and Sepharose 6B-ManαOMe were prepared as described (13). The columns (10 × 1.5 cm) were loaded with corn extract at 15 mL/h and washed with extraction buffer. Material was filtered through either column separately or serially. The retained proteins were eluted with 100 mM glycine-HCl buffer (pH 2.6). All fractions were analyzed for A_{280}. Fractions with the highest optical density were pooled, dialyzed against extraction buffer, and agglutinating activity was determined.

Lectin Affinity Chromatography

Two affinity columns (15 × 0.8 cm) were used, Sepharose 4B-Con A and Sepharose 4B-LCA, and washed with extraction buffer supplied with 1 mM each Ca^{2+} and Mg^{2+}. The retained glycoproteins were eluted with 3 M NH_4SCN. Fractions with highest A_{280} values were pooled and dialyzed against extraction buffer, and then agglutinating activity was determined.

Hemagglutination Assay

The test was carried out in small, round-bottom tubes at 37°C. Twofold serial dilutions of examined samples in the

[1] Abbreviations: LCA, *Lens culinaris* lectin; GalNAc, N-acetyl-D-galactosamine; ManαOMe, methylα-D-mannoside; PHA, *Phaseolus vulgaris* lectin; PNA, *Arachis hypogaea* lectin; PSA, *Pisum sativum* lectin; SBA, *Glycine max* lectin; WGA, *Triticum vulgaris* germ lectin; FPLC, fast protein liquid chromatography.

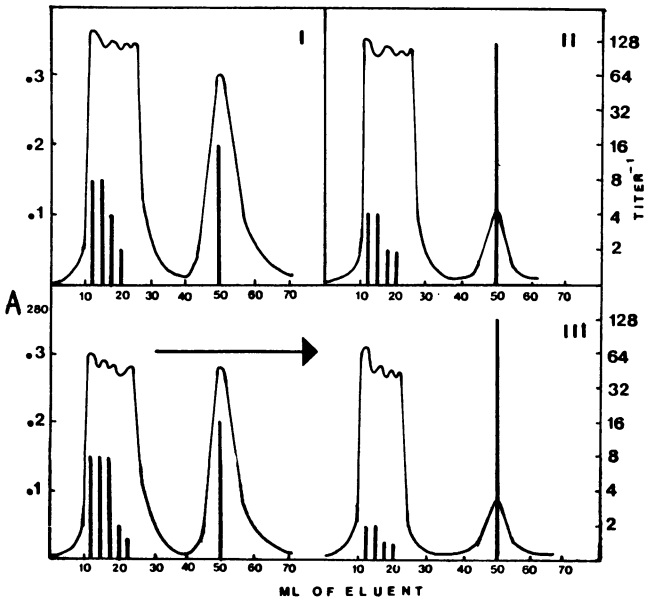

Figure 1. Biospecific affinity chromatography of corn extracts. I. Sepharose 6B-GalNAc. II. Sepharose 6B-ManαOMe. III. Serial filtration of corn extracts through Sepharose 6B-GalNAc followed by Sepharose 6B-ManαOMe. Columns were loaded with 10 mL crude extract each and eluted with 40 mL of 50 mM Tris-HCl (pH 7.5), and 30 mL of 100 mM glycine-HCl (pH 2.6). Hemagglutinating activity of rabbit erythrocytes is represented by vertical bars.

extraction buffer were mixed with equal volume (200 μL) of 2% suspension of untreated or trypsinized rabbit erythrocytes. Hemagglutination titer was observed after 1 h. The inhibitory effects of various saccharides were examined by preincubation of samples with sugar solutions at 37°C for 30 min, followed by hemagglutination assay.

FPLC

Affinity-purified lectins were submitted to FPLC on Superose 12 column, equilibrated with 50 mM Tris-HCl buffer (pH 7.5). Samples were loaded into a (1 × 30 cm) Superose 12 column via injection loops and were eluted at 0.3 mL/min. Effluents were monitored at 280 nm. Sample loads were 31 μg/100 μL and 44 μg/100 μL. Calibration standards consisted of blue dextran, 2×10^6; BSA, 67,000; ovalbumin, 43,000; hymotrypsinogen A, 25,000; ribonuclease A, 13,700 D.

SDS-PAGE

Subunit molecular mass was determined by SDS-PAGE on 15% sample gel and 3.75% stacking gels according to Laemmli (7). Samples containing pretreated bromphenol blue were pretreated by heating at 100°C, 5 min in 0.125 M Tris-HCl (pH 6.8), containing 2% SDS and 5% mercaptoethanol. After electrophoresis, the gels were stained by silver stain protocol according to Wray (12). The gels were calibrated with BSA, 66,000; ovalbumin, 44,000; glyceraldehyde 3-P dehydrogenase, 36,000; carbonic anhydrase, 29,000; trypsinogen, 24,000; trypsin inhibitor, 20,100, and α-lactalbumin, 14,200 D.

RESULTS AND DISCUSSION

Crude Extract of Salt-Soluble Proteins

The crude extract had weak agglutinating activity for rabbit (normal and trypsin-treated) but not for sheep erythrocytes. Besides, the crude extract was very unstable and had a tendency to form aggregates and became turbid during procedure. Therefore, it was impossible to determine lectin specificity at this stage by measuring inhibitory effects of various sugars on hemagglutinating activity.

An attempt to separate corn lectins from crude extract by $(NH_4)_2SO_4$ precipitation, which is often used for separation of other plant lectins, proved unsuccessful. After precipitation, reconstitution of precipitate, and dialysis of both fractions, agglutinating activity was distributed between precipitate and supernatant for all the examined saturations of $(NH_4)_2SO_4$ (20–80%).

Biospecific Affinity Chromatography

To determine lectin specificity and separate lectins from other proteins, affinity chromatography of crude extract was carried out in several columns bearing monosaccharides as biospecific ligands. Figure 1 (I, II) shows results of affinity chromatography of corn extracts on Sepharose 6B-GalNAc and Sepharose 6B-ManαOMe. Similar results were obtained using Gal and Man instead of GalNAc or ManαOMe, respectively.

Both biospecific affinity columns divided the loaded lectin materials into retained and nonretained portions as proven by agglutinating activity of the fractions. Sepharose 6B-GalNAc column retained more protein than Sepharose 6B-ManαOMe, but with a lower lectin activity. The nonretained portion had greater agglutinating activity when GalNAc was used as the specific ligand. Serial filtration of the material through both columns, however, was still insufficient to remove completely agglutinating activity from the filtrate (Fig.

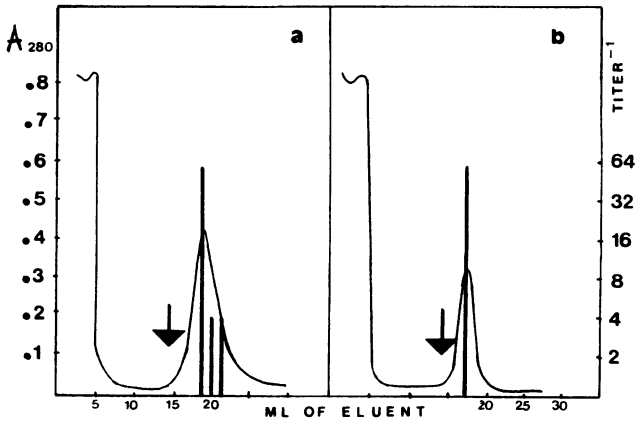

Figure 2. Lectin affinity chromatography of corn extracts. (a) Sepharose 4B-Con A; (b) Sepharose 4B-LCA. Columns were loaded with 2 mL crude extract each and eluted with 50 mM Tris-HCl buffer (pH 7.5), supplemented with 1 mM of each Ca^{2+} and Mg^{2+}, and then with 3 M NH_4SCN. Hemagglutinating activity is represented by vertical bars.

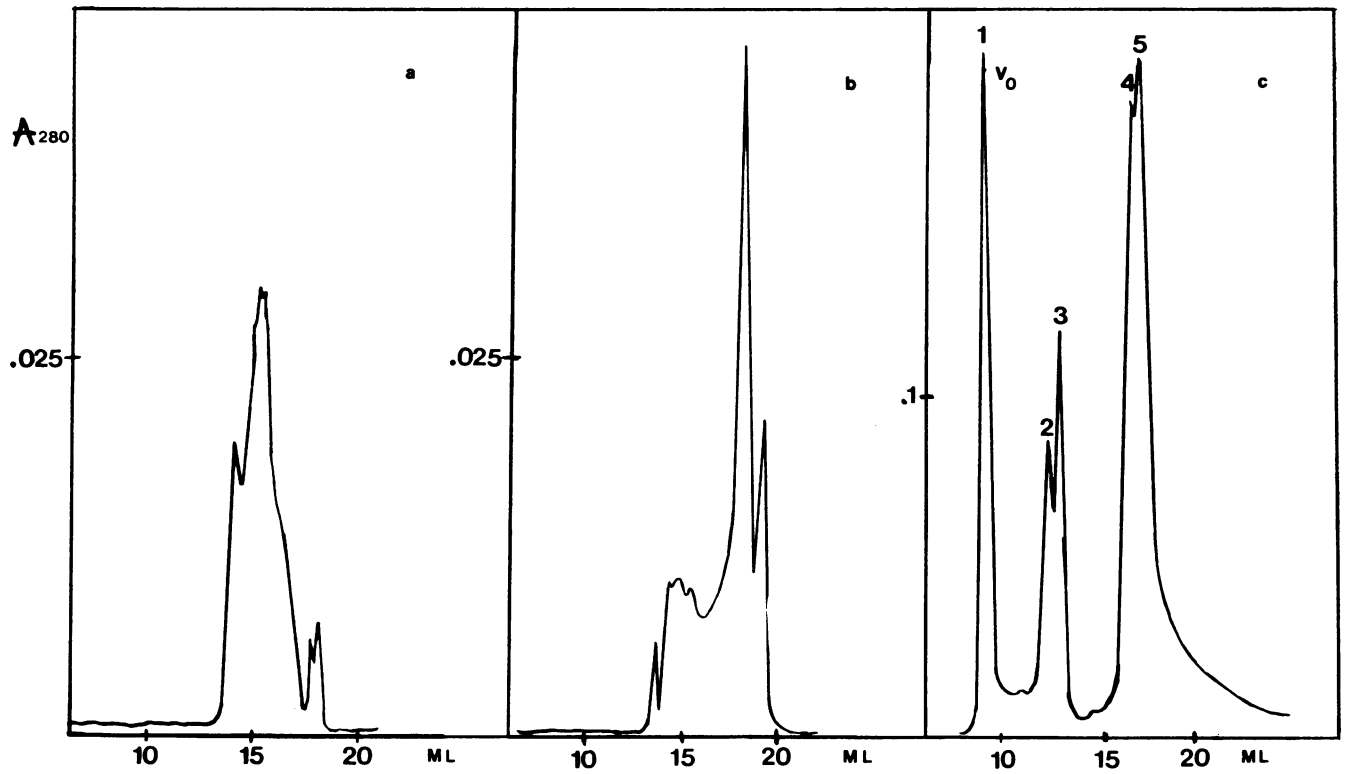

Figure 3. Superose 12 (1 × 30 cm column) FPLC of affinity-purified corn lectins. Samples were (a) ZMA-I; (b) ZMA-II; (c) calibration run: (1) blue dextran 2 × 10^6 D; (2) BSA 67,000 D; (3) ovalbumin 43,000 D; (4) hymotrypsinogen A 25,000 D; (5) ribonuclease A 13,700 D. Effluents were monitored at 280 nm. Flow rate was 0.3 mL/min.

1, III), but this was not the consequence of column saturation, as checked by rechromatography. Hemagglutinating activity that passed unbound through Sepharose 6B-GalNAc was inhibited mainly by Man, and of material that passed through Sepharose 6B-ManαOMe predominantly by Gal. The activity of material retained by the two columns was inhibited predominantly by sugars used as specific ligands.

The results indicate that corn salt-soluble proteins comprise at least two distinct types of lectins: GalNAc-interactive (ZMA-I) and Man-interactive (ZMA-II) lectin, and possibly some additional lectin activity with different specificity or a very weak affinity for the applied monosaccharides. Lepekhin and Ribak (8) also found a variation of carbohydrate-binding specificities of corn germ lectins and divided them accordingly into lactose-positive type (possibly corresponding to ZMA-I) and lactose-negative type (possibly ZMA-II). Neither of these lectins is immunochemically related to WGA or other cereal lectins. Affinity for Man is also a unique characteristic of ZMA, and has never been found before in lectins isolated from plants belonging to the Graminae family. The well-known Man-specific lectins (Con A, LCA, PSA) are all obtained from legume species. The taxonomical implications of these specificities of corn lectins remain to be examined.

Yields and Hemagglutinating Activity

The biospecific affinity columns retained 0.5 to 1.0% of the total loaded proteins of corn extracts. The elution of this fraction was more efficient when 100 mM glycine-HCl buffer (pH 2.6) was used instead of 0.5 M haptenic sugar solution. Nevertheless, the amount of bound and eluted protein was relatively small and amounted to 400 μg for Sepharose-GalNAc and 250 μg for Sepharose-ManαOMe from the same volume (15 mL) of corn extracts (average from 10 isolations). Yields calculated from these data would correspond to 13 mg ZMA-I and 8 mg ZMA-II/100 g of corn grain. However, the effective amounts of obtained lectins were considerably smaller due to the formation of turbidity and precipitation of

Table I. *Inhibitory Effects of Various Sugars on Hemagglutinating Activity of Corn Lectins Affinity-Purified on Sepharose 4B-Con A*

Starting concentration of all sugars was 500 mM with the exception of lactose (300 mM).

Sugar	Inhibition	Minimum Concentration Required to Inhibit Hemagglutination
		mM
Fructose	–	
Man	+	60
ManαOMe	+	60
Gal	+	250
GalNAc	+	250
Glucose	–	
N-Acetyl-D-glucosamine	–	
Lactose	–	
Sucrose	–	

proteins during storage of more or less purified preparations at 4°C, or freezing and thawing. Starting hemagglutinating activity of affinity purified corn lectins was 418 HU/mg ZMA-I and 2325 HU/mg ZMA-II, but it also decreased during storage.

Successive gel filtration on Bio-Gel P 100 of corn lectin preparations stored for 4 d at 4°C indicated that, besides the formation of precipitate, the proportion of soluble high molecular mass aggregates with low hemagglutinating activity constantly increased as the lectin activity of the preparation decreased.

The extracted corn lectins are inclined to form aggregates with other glycoconjugates present or autoaggregates, and the process proceeds continuously during storage. The result is an accumulation of high molecular mass aggregates with low activity and eventually a complete and irreversible loss of lectin activity. Such effects, extremely emphasized in corn, are also encountered in other Man-specific plant lectins, such as the lectins of *Canavalia ensiformis*, lentil, or pea. They considerably complicate lectin isolation and examination, and could be one of the technical reasons for as yet relatively scarce data on lectins in corn.

Lectin Affinity Chromatography

Structurewise, most of the known plant lectins are glycoproteins containing Man residue in their glycosidic moiety (3). Therefore, lectin affinity chromatography with two Man-specific lectins was investigated as the possible method of separation of ZMA population and study of their glycosidic structure.

The components showing lectin activity (Fig. 2) were wholly retained by Sepharose 4B-Con A, and eluted with 3 M NH_4SCN. Sepharose 4B-LCA column was also able to bind lectins from corn extract and release them upon elution with 3 M NH_4SCN as a sharp peak.

Agglutination of Con A affinity-purified corn extract was inhibited by sugars listed in Table I. Man and ManαOMe were the most potent inhibitors having effects at 60 mM concentration. Gal and GalNAc produced inhibitory effects at higher concentrations (250 mM plus). At concentrations of 500 mM or less, other sugars examined were inactive.

These results are likely to confirm that corn lectins are glycoproteins like many other lectins of plant or animal origin. According to the specificity of Con A (2), lectins separated from maize extract by affinity chromatography are bound to contain 3-mannosyl core with two α-bound Man residues. In addition, the affinity of corn lectins for LCA suggests that their glycosidic chain contains fucose residue also (6).

Molecular Mass and Subunits of ZMA-I and ZMA-II

FPLC revealed that both isolated salt-soluble corn lectins are composed of several fractions differing in molecular mass. ZMA-I preparation contains predominantly proteins with average M_r of 23,000 and small fractions of low M_r components corresponding to 12,500 and 11,500. The FPLC pattern of ZMA-II was reverse, consistent with highest proportion of M_r 12,500 fraction, followed by M_r 11,500 and small amounts of heavier components (Fig. 3). On SDS-PAGE, however, both lectin preparations appeared constituted by two subunits with molecular mass of 12,000 and 11,000. These data imply that mannose-interactive, salt-soluble corn lectin preparation, ZMA-II, contains mainly 12,500 subunit, while galactosamine-interactive ZMA-I comprises combinations of both subunits, possibly in the form of several isolectins differing slightly in their molecular mass, but with a common, predominating affinity for Gal-GalNAc.

It remains to be investigated whether the affinity for Man-ManαOMe or Gal-GalNAc is a distinct characteristic of each of the two identified ZMA subunits and the combination of the subunits determines the overall affinity of the corn lectin preparation, or the picture is even more complex.

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