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Analysis of the Lectins from Teosinte (*Zea diploperennis*) and Maize (*Zea mays*) Coleoptiles

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To identify molecular evidence of the common origin of maize and teosinte, a lectin from teosinte coleoptile (TCL) was purified, through affinity chromatography on a lactosyl-Sepharose column, and some of the physicochemical parameters were compared with those from the maize coleoptile lectin (CCL). TCL is a 92 kDa glycoprotein constituted mainly by aspartic, glutamic, glycine, leucine, and lysine residues; in minor proportion, methionine and cysteine were also found. The glycannic portion of the lectin, which corresponds to 10% w/w, is composed by Gal, Man, and GlcNAc. CCL is an 88.7 kDa glycoprotein that contains 12% sugars by weight; its sugar and amino acid compositions are similar to those of TCL. TCL is formed by two isoforms identified through acidic electrophoresis, whereas CCL is constituted by a single molecular form. The NH_2 termini of both TCL isoforms are blocked, but their amino acid sequences determined from tryptic peptides by matrix-assisted laser desorption ionization time-of-flight) indicated that TCL isoforms have no homology with other mono- or dicotyledonous lectins, including CCL. TCL, just as CCL, showed hemagglutinating activity toward animal erythrocytes, including human A, B, and O. Hapten inhibition assays indicated that although TCL shows broader sugar specificity than CCL, it recognizes Gal in O- and N-glycosidically linked glycans. Both lectins are equally well recognized by antibodies against TCL.

KEYWORDS: Plant lectins; Gramineae lectin; galactose specific lectin; glycoproteins; *Zea mays*; *Zea diploperennis*; maize; teosinte

INTRODUCTION

Lectins, due to their great capacity to bind specifically carbohydrates (*1*), have been shown to be useful tools to recognize glycosylated antigens and markers in different tissues and cells (*1*−*4*). At present, many biological activities have been evidenced in lectins (*3*, *4*), and several studies support the participation of plant lectins in the specific interaction with rhizobial bacteria (Rhizobium and Bradyrrhizobium) (*3*−*5*). It has been also suggested that lectins from some plant species participate in defense mechanisms, due to their capacity to inhibit protein synthesis (*6*). Some plant lectins possess antiviral and bactericidal activity against plant pathogens (*7*−*10*).

In maize (*Zea mays*), recent findings evidenced the presence of an 88.7 kDa β-galactose-specific lectin, identified exclusively in the emerging coleoptile (*11*, *12*). It has been suggested that maize is the product of homeotic sexual translocations of teosinte (*Zea diploperennis*) (*13*); however, other studies support the notion that gene duplication is an active process in the evolution of the maize genome. On the other hand, it has long been suspected that maize is the product of a tetraploid event promoted by two diploid progenitors: maize and teosinte (*14*). Several authors have proposed that changes in a small number of regulatory genes may be sufficient for the evolution of novel morphologies and that some teosinte genes played such a role during the morphological evolution of maize from its wild ancestor (*15*). Teosinte and maize are members of the Andropogoneae tribe, and despite their morphological differences, evidence suggests that teosinte's evolution was favored by human selection and by stepwise homeotic sexual conversions, explaining in part both archaeological and morphological findings and external characteristics (*13*). These findings suggest that lectins from both plants show similarities and support the hypothesis that maize could be the product of teosinte's evolution. We purified a lectin from the coleoptile of teosinte and performed its chemical characterization. The amino acid sequence of the lectin from teosinte was analyzed from trypsin-digested glycopeptides through matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF), and its specificity for sugar residues was compared with that from maize (corn) coleoptile lectin.

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MATERIALS AND METHODS

Seeds and Germination. Teosinte (*Z. diploperennis*) var. Oaxaca and maize (*Z. mays*) seeds were obtained from the Central Valley of Oaxaca and classified by INIFAP-Zacatepec, Morelos (*12*). The seeds were superficially sterilized with 0.5% $HgCl_2$ in water for 7 min at 25 °C under vigorous shaking; afterward, they were washed with distilled water and germinated in filter papers (Ederol) at 25 °C for several time intervals in the dark.

Extraction and Isolation of TCL. Teosinte or maize coleoptiles were excised with a blade at daily time intervals up to the 10th day of culture; 500 g of coleoptile was homogenized separately with 500 mL of 0.1 M citrate buffer, pH 6.4, under continuous stirring at 4 °C for 5 min. The supernatant was filtered through Whatman no. 1 filter paper containing 1 g of activated charcoal. The clear filtrate (crude extract) was tested for hemagglutinating activity and for protein concentration (see further). Coleoptile crude extracts were precipitated with 66% cold acetone; then, the precipitated proteins were extracted and suspended in citrate buffer, pH 6.4 (*17*), and the acetone precipitated extract obtained from coleoptiles was poured onto a column (2 × 20 cm) containing lactosyl-Sepharose 4B (Sigma Fine Chemicals, St. Louis, MO), previously equilibrated with citrate buffer, at 4 °C at a flow rate of 12 mL/h. The lectin from the teosinte coleoptile was eluted with 0.2 M lactose in citrate buffer and that from corn coleoptile was eluted with 0.15 M lactose (*12*). The fractions (1.5 mL each) were measured for OD at 280 nm and for hemagglutinating activity in the presence of human erythrocytes (A, B, or O type). The lectins in citrate solution with 5% glycerol were stored in the cold cabinet for further analyses.

Analytical Methods. Protein concentration was determined according to the method of Bradford (*18*), using bovine serum albumin as standard. The homogeneity and molecular weight of the purified TCL were determined through electrophoresis on polyacrylamide gel under non-denaturing conditions in alkaline pH (8.3) phosphate buffer, according to the method of Hedrick and Smith (*19*). Briefly, TCL was electrophoresed at 5, 7.5, 10, and 12.5% polyacrylamide concentrations, and the R_f relative to the tracking dye (bromophenol blue) was determined in each gel and plotted against the percentage of gel concentration. From these plots, the individual slope was determined for TCL and the logarithm of the negative slope was plotted against the logarithm of the molecular weight of control proteins. Homogeneity of TCL was also analyzed through polyacrylamide gel electrophoresis under non-denaturing conditions, at 4 °C, with 0.1 M β-alanine buffer and 1 M urea, pH 4.5, according to the method of Reisfeld et al. (*20*) with a 7% acrylamide and 0.4% bisacrylamide resolving gel and methyl green as the tracking dye, at a current of 40 mA slab for 12 h. Finally, TCL and CCL purified lectins were analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (*21*).

Amino Acid Analysis. Amino acid composition of TCL was determined in 100 μg of lectin hydrolyzed under vacuum with 2 mL of 6 M HCl at 110 °C in sealed tubes for 24, 48, and 72 h. The samples were analyzed in an automatic amino acid analyzer Durrum 500, according to Bidlingmeyer et al. (*22*), using norleucine as internal standard. The amino acid sequence was determined in the purified TCL on a Beckman Instruments (Fullerton, CA) model LF3000 protein sequencer interfaced with an Applied Biosystems model 1120 on-line analyzer. Amino acid sequencing of TCL was also analyzed by MALDI-TOF in peptide fragments obtained by trypsin digestion of the purified lectin. After electrophoresis in acidic conditions of the purified lectin (*19*), bands were excised with a scalpel, and each band containing 200 pM of protein was digested with 0.5 μg of trypsin (Promega sequencing grade) in 500 μL of ammonium bicarbonate, pH 8.0, at 37 °C, for 24 h. The enzyme digest was then evaporated to dryness using a Speed Vac concentrator (Savant Instruments Inc., Holbrook, NY) and re-evaporated with water (2 × 100 μL). Samples were prepared by mixing, directly onto the target, 1 μL of the reaction products containing 50 pM of protein and 1 μL of a 2,5-dihydroxybenzoic acid matrix (12 mg/mL in acetonitrile/H_2O, 80:20, v/v), allowing the mixture to crystallize at room temperature (22 ± 3 °C). Positive ions of the peptides were measured by MALDI-TOF on a Vision 2000 time-of-flight mass spectrometer (Finnigan MAT, Bremen, Germany) equipped with a 337 nm UV laser. Mass spectra were acquired in reflectron mode under an 8 keV acceleration voltage and positive detection. Control assays were performed using trypsin alone to identify self-digested peptide mass and with angiotensin I as standard (M_r 1296.7) (*23*). The mass of $[M + H]^+$ ions from peptides produced by tryptic digestion was compared with those obtained from the NCBInr (Swiss-Prot 05/05/2001) database.

Hemagglutination Assays. The hemagglutinating activity was tested in microtiter U plates (NUNC, Roskilde, Denmark) according to a 2-fold serial dilution procedure with either 2% (w/v) untreated erythrocyte suspension in phosphate-buffered saline (PBS; 0.01 M sodium phosphate, 0.15 M sodium chloride, pH 7.2) or sialidase-treated (*Vibrio cholerae* fraction V; 0.1 U/0.5 mL of packed erythrocytes at 37 °C for 30 min) or Pronase-treated (*Streptomyces griseus* protease fraction XXV; 100 μg/0.5 mL of packed erythrocytes at 37 °C for 30 min) red blood cells. The hemagglutinating titer is reported as the inverse of the last dilution with agglutinating activity.

Preparation of Glycoproteins. Glycoproteins were desialylated by incubation at 100 °C for 1 h in the presence of 0.02 N sulfuric acid, as described by Spiro and Bhoyroo (*24*), and desalted on a Bio-Gel P-2 column (2 × 60 cm) equilibrated with 0.5 M acetic acid. β-Galactosidase digestion of asialo-glycoproteins was performed at 37 °C in 0.02 M citrate buffer, pH 3.5, for 12 h with 10 mU of jackbean β-galactosidase for each 100 μM of glycan as described by Spik et al. (*25*). Then, the asialo-agalacto-glycoproteins were desalted on a Bio-Gel P2 column equilibrated with water and lyophilized until use. The *O*-glycan (Galβ1,3GalNAc) from fetuin was a gift from Prof. Henri Debray, Université des Sciences et Technologies de Lille, France. The carbohydrate composition of glycoproteins used in this study as well as of the purified TCL and CCL was determined with the heptafluorobutyrate derivatives of *O*-methyl-glycosides from the lectin and its glycopeptides, obtained after methanolysis in 0.5 M methanol−HCl for 24 h at 80 °C; lysine (Sigma) was used as internal standard. Samples were analyzed by gas chromatography using a capillary column (25 × 0.32 mm) of 5% silicone OV 210 (Applied Science Laboratories, Buffalo, NY), in a Varian 2100 gas chromatograph (Orsay, France) (*26*).

Sugar Specificity. The sugar specificity of the lectins was tested by inhibiting the hemagglutinating activity with simple sugars, glycosides, and glycoproteins. The lectins diluted in PBS to a hemagglutinating titer of 4 (4 hemagglutinating dose units) were incubated at room temperature for 1 h with different concentrations of inhibitors before the addition of 2% human erythrocytes type 0 in PBS. Results are expressed as the minimal concentration of carbohydrates and glycoproteins with well-known structure that effectively inhibited 4 hemagglutinating dose units of the lectin (*27*).

Rabbit IgG Anti-TCL. Two-month-old female New Zealand rabbits were immunized intraperitoneally, with 300 μg of teosinte purified lectin in 1 mL of Freund's complete adjuvant; then, 100 μg booster injections in 500 μL of Freund's incomplete adjuvant were given intraperitoneally at 2-week intervals. A final intravenous injection with 100 μg of lectin in saline was given 2 days before the rabbit was bled. Blood was obtained by cardiac puncture, and immunoglobulin G (IgG) was purified by fractionation with 50% solid ammonium sulfate and ionic exchange chromatography on DEAE-Sephadex (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). Double-immunodiffusion was performed in 1.5% agar/PBS plates according to Ouchterlony's procedure.

RESULTS

Purification of TCL. Using teosinte coleoptile extracts, we purified a lectin (TCL) by precipitation of the crude extract with cold acetone and affinity chromatography on a lactosyl-Sepharose 4B column, and it was recovered from the column by the addition of 200 mM lactose (**Figure 1**). Results indicated that, as for the lectin from maize, the optimal concentration of teosinte lectin was obtained on the seventh day of germination. After this time, the lectin concentration decreased to low hemagglutinating activity. As summarized in **Table 1**, the purification process increased 1076-fold the teosinte lectin-specific activity, corresponding to 0.07% of the total weight.

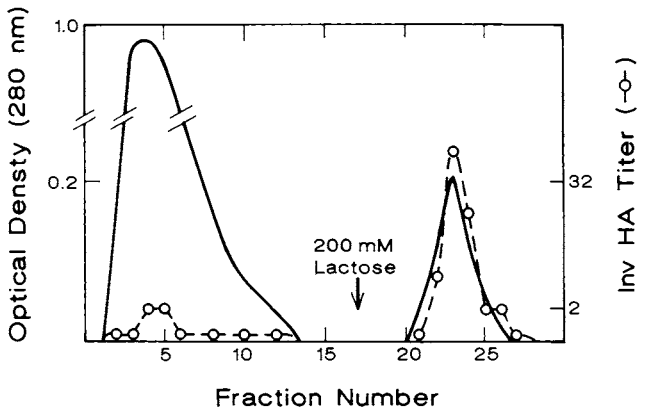

Figure 1. Purification process of teosinte coleoptile lectin by affinity chromatography. Coleoptile extract (50 mg of protein) after precipitation with 66% cold acetone and suspension in citrate buffer was poured onto a column (2 × 20 cm) containing lactosyl-Sepharose 4B in 0.1 M citrate buffer, pH 6.4. Elution of the lectin was performed with 200 mM lactose in citrate at 4 °C, and the fractions (1.5 mL each) were dialyzed against citrate buffer before testing of their OD at 280 nm and hemagglutinating activity in the presence of 2% human erythrocytes type A in PBS.

Table 1. Purification Process of the Teosinte Coleoptile Lectin on Lactosyl-Sepharose 4B[a]

fraction	HA activity (UHA)	protein (mg)	specific activity (UHA/mg of protein)
crude	13×10^6	2×10^3	6.5×10^3
66% cold acetone	10×10^6	530	18.8×10^3
lactosyl-Sepharose	10.1×10^6	1.42	7×10^6

[a] From 500 g of teosinte coleoptile.

Purification of maize (corn) coleoptile lectin (CCL), under similar conditions, increased 1785-fold the activity, with a yield of 0.05% of the initial weight.

Chemical Characterization. Homogeneity of the purified fraction was determined by polyacrylamide electrophoresis in alkaline (pH 8.3) conditions, and as indicated in **Figure 2b** a single band is observed. Electrophoresis performed in acidic (pH 4.5) buffer, under non-denaturing conditions, renders two bands, further termed TCL isoforms a and b (**Figure 2c**). The molecular mass of the maize and teosinte lectins was determined according to the procedure of Hedricks and Smith (*19*), and our results showed that both types of lectins have close molecular weights: 88.7 kDa for maize and 92 kDa for teosinte lectin (**Figure 3**). SDS-PAGE analysis of the purified TCL and CCL revealed similar electrophoretic patterns with five subunits of 60, 48, 42, 36, and 31 kDa (**Figure 4**).

The amino acid composition of TCL is listed in **Table 2**. Similarly to the corn coleoptile lectin, TCL is rich in aspartic, glutamic, glycine, leucine, and lysine residues and poor in methionine, cysteine, and histidine residues (**Table 2**). TCL and CCL are glycoproteins containing 10 and 12% w/w of sugars, respectivly, and composed by Gal, Man, and GlcNAc residues (**Table 3**). The amino terminus determined in TCL by an amino acid sequencer, using Edman degradation, indicated that the amino residue is blocked; for this reason, we determined the amino acid sequence of the two isoforms of the purified lectin by analyzing the peptides obtained after acidic electrophoresis through trypsin digestion by MALDI-TOF and comparing them with the relative values obtained from the NCBInr database. We obtained 23 peptides from TCL isoform a and 38 peptides

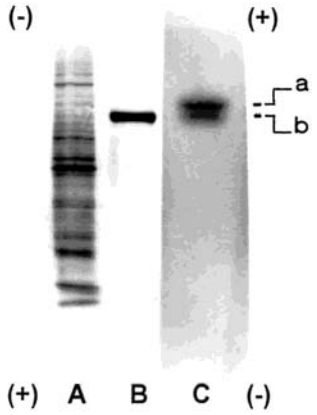

Figure 2. Polyacrylamide gel electrophoresis of the purified teosinte coleoptile lectin: (lane A) teosinte crude extract (35 μg); (lane B) teosinte coleoptile lectin (5 μg) electrophoresed in alkaline non-denaturating conditions, pH 8.3; (lane C) teosinte coleoptile lectin (5 μg) electrophoresed in acid conditions, pH 4.5.

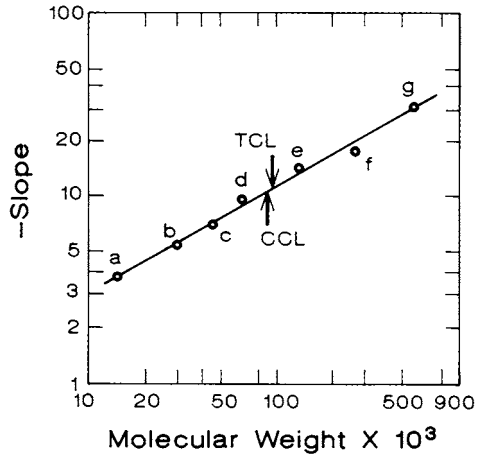

Figure 3. Comparative molecular weights of teosinte (TCL) and corn (CCL) coleoptile lectins by polyacrylamide gel electrophoresis under non-denaturating conditions. Electrophoreses on gels at 5, 7.5, 10, and 12.5% polyacrylamide concentrations, at pH 8.3, were performed, and the relative R_f of the protein in each gel relative to the tracking dye (bromophenol blue) was plotted. Molecular weight standards were (a) α-lactalbumin (14200), (b) carbonic anhydrase (29000), (c) ovalbumin (45000), (d and e) bovine serum albumin (monomer, 66000; and dimer, 132000), and (f and g) urease (trimer, 272000; and hexamer, 545000).

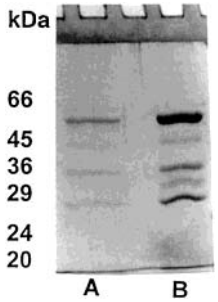

Figure 4. SDS-PAGE of the purified TCL (A) and CCL (B) lectins. The molecular weight standards used were bovine serum albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), chymotrypsinogen A (25 kDa), and trypsin inhibitor (20 kDa).

from TCL isoform b; their masses ranged from 614.1 to 3637.5 (MH^+). As indicated in **Table 4**, the 10 tryptic peptides obtained

Table 2. Amino Acid Composition of Teosinte[a] and Corn Coleoptile Lectin[b]

	teosinte lectin		corn lectin	
amino acid	res/%	res/mol	res%	res/mol
Asx	10.8	81	10.1	79
Glx	9.3	70	11.1	87
Ser	6.7	49	8.2	64
Gly	9.6	73	11.4	89
His	1.4	18	1.9	15
Arg	2.3	40	3.1	24
Thr	5.4	57	4.7	37
Ala	7.6	53	7.3	57
Pro	7.1	49	5.1	40
Tyr	1.8	22	3.0	23
Val	5.6	61	5.6	44
Met	1.2	9	2.7	21
Cys	0.4	3	0.7	5
Ile	5.2	39	4.2	33
Leu	10.2	77	8.5	66
Phe	7.0	52	5.4	40
Lys	8.6	64	7.0	55

[a] Calculated on the basis of 92 kDa and considering that 10% weight corresponds to carbohydrates. [b] Calculated on the basis of 88.7 kDa and considering that 12% weight corresponds to carbohydrates (data from ref *12*).

Table 3. Carbohydrate Composition of the Teosinte and Corn Coleoptile Lectin

carbohydrate	res/%	res/mol[a]	res/%	res/mol[b]
Gal	42	21	34	24
Man	21	11	19.3	14
GlcNAc	37	15	46.7	27

[a] Data calculated based on 92 kDa and considering that carbohydrate content corresponds to 10% of the lectin's total weight, respectively. GalNAc, fucose, xylose, rhamnose, and glucuronic acid were not identified. [b] Data calculated based on 88.7 kDa and considering that carbohydrate content corresponds to 12% of the lectin's total weight, respectively. GalNAc, fucose, xylose, rhamnose, and glucuronic acid were not identified.

Table 4. Predicted Amino Acid Sequence from Tryptic Peptides of the Subunit of Teosinte Coleoptile Lectin Isoform A, Determined by MALDI-TOF[a]

monoisotropic mass + 1	amino acid position	peptide sequence	related protein[b]
1349.46	21–31	FTFDEIYDATK	PKA
1308.89	45–55	FNKDLCLQLSK	A*
1349.39	134–144	RDFVIINFVAR	A*
1792.49	165–179	SIVYIEPPIIHRDIK	PKA
1309.44	180–190	SSNILLTENIR	PKA
2590.49	199–221	PIYPLIHPAVLVETYEHGESVAR	A*
1670.89	301–314	VLEMAFQCLAPHRR	PKA
875.09	312–319	RDGRTAAER	A
1192.59	394–403	KLDPGYDVMR	A
1267.49	417–428	SLSYTIDGLMAP	A

[a] Molecular ions from the LS/MS spectrum of tryptic digest of 20 pmol of lectin were identified by comparing their relative values with those obtained from the NCBInr (05/05/2001) database. The band was obtained from acidic electrophoresis (pH 4.5). [b] Matched proteins with the highest score correspond to (A) a 49 kDa protein from *Arabidopsis thaliana* (28% of homology) and (PKA), a 41 kDa protein from *A. thaliana* (22% homology). An asterisk (*) indicates homology with the teosinte coleoptile lectin isoform b. The NH_2 terminal is blocked. The assigned amino acid position corresponds to the peptide sequence of the homologous protein.

from isoform a of the purified TCL showed 28% homology with a 49 kDa protein from *Arabidopsis thaliana* and 22% homology with a 41 kDa, hypothetical, protein from *A. thaliana*.

Table 5. Predicted Amino Acid Sequence from Tryptic Peptides of the Teosinte Coleoptile Lectin Isoform B, Determined by MALDI-TOF[a]

monoisotropic mass + 1	amino acid position	peptide sequence	related protein[b]
2053.9	1–19	-REILHIQGGQCGNQIGAK	T
1702.16	1–17	MCGILVAVLGVVEVSLAK	Z
1765.9	3–19	EILHIQGGQCGNQIGAK	T
2590.29	36–58	YAGDSDLQLERINVYYNEASGGR	T
1309.19	45–55	FNKDLCLQLSK	A*
1342.43	47–58	INVYYNEASGGR	T
2133.49	59–77	FVPRAVLMDLEPGTMDSVR	T
893.19	71–78	SIENAFGR	A
1020.39	70–78	KSIENAFGR	A
2660.69	79–102	KLSEIFEEFDEAPVASGSIAQVHR	A
1350.39	134–144	RDFVIINFVAR	A*
2590.29	199–221	PIYPLIHPAVLVETYEHGESVAR	A*
1334.99	234–246	AKVAHIGTNALLK	A
1716.29	323–337	LSKQQNCPDPQAFIK	A
2249.19	338–357	MVISGEGSDEIFGGYLYFHK	Z
2660.69	338–361	MVISGEGSDEIFGGYLYFHKAPNK	Z
1361.19	383–395	ANKATSAWGVEAR	Z
933.09	505–512	RFPQDSAR	Z
1611.29	563–578	WPTAAARPANGTVNGK	Z

[a] The molecular ions from the LS/MS spectrum were identified as indicated in **Table 4**. [b] Matched proteins with the highest score correspond to (A) a 49 kDa protein from *A. thaliana* (28% of homology); (T) is a 58 kDa, β-tubulin 4 from *Triticum aestivum* (17%), and (Z) is a 67 kDa asparagine synthetase from *Z. mays* (18% homology). The NH_2 terminal is blocked (–). An asterisk (*) indicates homology with teosinte coleoptile lectin isoform a.

Table 6. Hemagglutinating[a] Activity of Teosinte Coleoptile Lectin[b]

		enzyme treatment	
erythrocyte	native	pronase	sialidase
human A	8	512	1024
human B	16	1024	2048
human O	16	1024	2048
rabbit	8	2048	4096
rat	4	256	512
mouse	4	32	64
sheep	2	64	128

[a] The hemagglutinating titer is reported as the inverse of the last dilution with agglutinating activity. [b] The lectin concentration was 250 μg/mL. Pronase was from *S. griseus* and sialidase from *V. cholerae*.

After trypsin treatment of the TCL isoform b, 19 peptides matched with three proteins, which correspond to a 49 kDa protein from *A. thaliana* (28% homology), a 58 kDa β-tubulin 4 from *Triticum aestivum* (17%), and a 67 kDa asparagine synthetase from *Z. mays* (18% homology) (**Table 5**). It is interesting to note that only three peptides with *m*/*z* of 1308, 1349, and 2590, corresponding to a 49 kDa protein from *A. thaliana*, were common for the two TCL isoforms (**Tables 4** and **5**).

Hemagglutinating Activity. TCL agglutinated erythrocytes from the animal species tested. Its highest hemagglutinating capacity was observed for human erythrocyte type O, which was higher than for human erythrocyte types B and A. Pronase or neuraminidase treatment of red blood cells increased 6−7-fold the hemagglutinating activity of the lectin, and in rabbit erythrocytes the treatment increased 8−9-fold the hemagglutinating activity; TCL showed the lower agglutinating activity in the presence of rat, mouse, and sheep erythrocytes (**Table 6**).

Sugar Specificity. TCL's sugar specificity was determined by hemagglutinating inhibition assays (**Table 7**). Galactose and lactose at 200 mM and sialyllactose (α-2,3- or α 2,6-siallyl-

Table 7. Inhibition of Hemagglutination of TCL and CCL by Sugars and Glycoproteins[a]

inhibitor	concentration (mM) TCL	CCL
D-Gal	200	150
Galβ1,4Glc	200	75
Galβ1,4GlcNAc	100	75
NeuAcα2,3Galβ1,4GlcNAc	100	75
NeuAcα2,6Galβ1,4GlcNAc	100	75
Galβ1,3GalNAc	100	75
α-methyl-Gal	100	75
β-methyl-Gal	100	25
porcine stomach mucin	0.0001	0.00001
ovine submandibular mucin	0.01	0.003
asialo ovine mucin	0.005	0.001
bovine submandibular mucin	0.001	0.0003
bovine asialo-mucin	0.0005	0.0001
calf fetuin	0.0005	0.00005
asialo-fetuin	0.0001	0.00001
asialo-agalacto-fetuin	NI	NI
human α_1-acid glycoprotein	0.01	0.01
asialo-α_1-acid glycoprotein	0.002	0.005
asialo-agalacto-α_1-acid glycoprotein	NI	NI

[a] Results are represented as the minimal concentrations required to inhibit the hemagglutination activity of TCL and CCL (titer = 4). D-Gal, D-Glc, D-Man, L- and D-Fuc, D-GlcNH$_2$, D-GalNH$_2$, sucrose, D-ribose, D-Fruc, D-sorbitol, melibiose, D-mannitol, maltose, maltotriose, D-arabinose, D-Xyl, melezitose, D-celobiose, L-rhamnose, GlcNAc, and GalNAc did not inhibit at 300 mM. Chicken ovalbumin and turkey ovalbumin were not inhibitory (NI) at a 0.01 mM concentration.

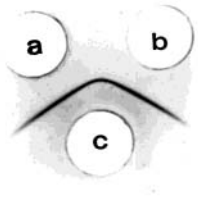

Figure 5. Double-immunodiffusion plate of polyclonal rabbit antibodies against teosinte coleoptile lectin in 1.5% agar: (a) teosinte (10 μg); (b) corn coleoptile lectin (10 μg); (c) central well, rabbit antibodies.

lactose) as well as *N*-acetyllactosamine, at 100 mM, inhibited 4 hemagglutinating units of TCL. Other monosaccharides, including sialic acid, failed to inhibit TCL's hemagglutination capacity even at a 300 mM concentration. Methyl α- or β-galactosides were as good inhibitors as galactose, indicating that TCL binding is not influenced by the galactose anomeric carbon; however, for CCL, the methyl-β-anomer of galactose inhibited 3 time more powerfully its hemagglutinating activity than the α-anomer. TCL and CCL's hemagglutinating activities were effectively inhibited by glycoproteins, such as fetuin, α_1-acid glycoprotein, ovine and bovine submandibular mucins, and porcine stomach mucin. Hen and turkey ovalbumins showed no inhibitory capacity for CCL at 10 μM concentrations. TCL always required higher concentrations of sugars or glycoproteins than CCL to inhibit its hemagglutinating activity. Elimination of sialic acid residues from the inhibitory glycoproteins increased the interaction with the lectins 5−10 times, whereas elimination of galactose residues from fetuin and α_1-acid glycoprotein abolished their capacity to inhibit the lectins' activity.

Immunological Characterization. The antiserum, from rabbits immunized with TCL, when examined in double immunodiffusion agar plates against teosinte and corn lectin yielded a single continuous immunoprecipitation broad band (**Figure 5**).

DISCUSSION

Gene duplication has been considered to be an active process in the evolution of the maize genome. It has long been suspected that maize is the product of a historical tetraploid event; the diploid progenitors diverged roughly 20.5 million years ago, whereas the allotetraploid event probably occurred approximately 11.4 million years ago (*14*, *28*). Other evidence suggests that teosinte, through homeotic sexual translocations, gave rise to maize (*13*). In this work we purified the lectin from teosinte coleoptile and compared it with corn coleoptile lectin to identify their possible common evolutionary origin.

Similarly to CCL, TCL agglutinates erythrocytes from human and other animal species equally well; although sialidase or protease treatment enhanced the hemagglutinating activity, all of the erythrocytes tested were well agglutinated, indicating that the specificity of TCL, similarly to CCL (*12*, *17*), is not addressed to blood group determinants. Both lectins showed specificity for galactose residues, and this specificity seems to be addressed mainly to the OH group of C4; the anomeric C1 from the same residue seems to be irrelevant for the accommodation of the TCL receptor, because α- and β-anomers were equally well recognized by TCL in contrast to CCL, which recognizes preferentially β-anomers. Lactose (Galβ1,4Glc), and *N*-acetyllactosamine (Galβ1,4GlcNAc) inhibited the lectin's activity. Specificity for galactose residues allowed these lectins to interact with O-glycosidically linked glycans containing Galβ1,3GalNAc α1,0 Ser/Thr and GalNAc α1,0 Ser/Thr present in fetuin (*24*), ovine (*31*), and bovine (*32*) submandibular mucins or pig stomach mucin (*27*). TCL hemagglutinating activity is inhibited by human α_1-acid glycoprotein (*33*) and fetuin, which also contain tri- and tetra-antennary N-glycosidically linked glycans of *N*-acetyllactosaminic type (*24*). Hen ovalbumin (*25*) and turkey transferrin (*34*), containing oligomannosidic N-glycosidically linked glycans, showed no inhibitory capacity for TCL and CCL. Elimination of sialic acid from the inhibitory glycoproteins enhanced the interaction of CCL and TCL with their corresponding receptors by exposing cryptic galactose residues; however, galactosidase treatment abolished the inhibitory capacity of glycoproteins, confirming the relevance of galactose interactions of both lectins with their receptor, although it is clear that TCL seems to possess a broader capacity to recognize galactose residues than CCL, because higher concentrations of galactose-containing structures were necessary to inhibit TCL's activity.

Duplication events give rise to close isoforms in a single species, which could be a mechanism to control specific expressions according to developmental and environmental conditions (*15*, *35*). Along maize's evolution, which has been favored by human selection during horticultural domestication (*13*), some protein domains with the lectin from teosinte seem to have been preserved, because both lectins showed crossed immunogenicity together with sugar specificity. Lectins have evolved through gene duplication and divergence, and their carbohydrate-binding domains have become incorporated into the family of proteins (*6*, *9*, *36*). Maize and teosinte belong to the Gramineae group; they showed specificity for galactose, differing from lectins of the same group (such as WGA, rice, and rye, among others), and most of them recognize GlcNAc and its oligomers (*37*−*39*), suggesting the possibility that maize and teosinte emerged from a common precursor and have evolved assisted by ancient human agriculture. A recent publication (*40*), using multilocus microsatellite analysis of *Zea* species, suggested that maize originated from the domestication of *Zea parviglumis.* This most recent finding could well explain

the differences in amino acid sequences between *Z. mays* and *Z. diploperennis* found in this work.

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LITERATURE CITED

(1) Sharon, N.; Lis, H. Legume lectins, a large family of homologousproteins. *FASEB J.* **1990**, *4*, 3198−208.

(2) Lascurain, R.; Porras, F.; Báez, R.; Chávez, R.; Martínez, S.; Alvarez, G.; Zenteno, E. *Amaranthus leucocarpus* lectin recognizes human naive T cell subpopulations. *Immunol. Invest.* **1997**, *23*, 579−587.

(3) Slifkin, M.; Doyle, R. J. Lectins and their application to clinical microbiology. *Clin. Microbiol. Rev.* **1990**, *3*, 197−218.

(4) Van Damme, E. J.; Peumans, W. J.; Barre, A.; Rouge, P. Plant lectins: A composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. *Crit. Rev. Biochem. Mol. Biol.* **1998**, *33*, 209−258.

(5) Diaz, C. L.; Spaink, H. P.; Kijne, J. W. Heterologous rhizobial lipochitin oligosaccharides and chitin oligomers induce cortical cell divisions in red clover roots, transformed with the pea lectin gene. *Mol. Plant Microbe Interact.* **2000**, *13*, 268−276.

(6) Chrispeels, M. J.; Raihhel, N. V. Lectins, lectin genes, and their role in plant defense. *Plant Cell* **1991**, *3* (1), 1−9.

(7) Kumar, M. A.; Timm, D. E.; Neet, K. E.; Owen, W. G.; Peumans, W. J.; Rao, A. G. Characterization of the lectin from the bulbs of *Eranthis hyemalis* (winter aconite) as an inhibitor of protein synthesis. *J. Biol. Chem.* **1993**, *268*, 25176−25183.

(8) Rao, K. V.; Rathore, K. S.; Hodges, T. K.; Fu, X.; Stoger, E.; Sudhakar, D.; Williams, S.; Christou, P.; Bharathi, M.; Bown, D. P.; Powell, K. S.; Spence, J.; Gatehouse, A. M.; Gatehouse, J. A. Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistance to rice brown plant-hopper. *Plant J.* **1998**, *15*, 469−477.

(9) Drickamer, K. Making a fitting choice: common aspects of sugar-binding sites in plant and animal lectins. *Structure* **1997**, *5*, 465−478.

(10) Pérez-Santiago, A.; Saavedra, E.; Pérez-Campos, E.; Córdoba, F. Effect of plant lectins on *Ustilago maydis in vitro*. *Cell Mol. Life Sci.* **2000**, *57*, 1986−1999.

(11) Martinez-Cruz, M.; Cordoba, F. Isolation of fully active and stable teosinte coleoptile lectins. *Prep. Biochem. Biotechnol.* **2000**, *30*, 199−208.

(12) Martínez-Cruz, M.; Zenteno, E.; Córdoba, F. Purification and characterization of a galactose-specific lectin from corn (*Zea mays*) coleoptile. *Biochim. Byophys. Acta* **2001**, *1568*, 37−44.

(13) Iltis, H. H. Homeotic sexual translocations and the origin of maize (*Zea mays*, Poaceae): A new look at an old problem. *Econ. Bot.* **2000**, *54*, 7−42.

(14) Gaut, B. S.; Doebley, J. F. Evolution DNA sequence evidence for the segmental allotetraploid origin of maize. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6809−6814.

(15) Lukens, L.; Doebley, J. Molecular evolution of the teosinte branched gene among maize and related grasses. *Mol. Biol. Evol.* **2001**, *18*, 627−638.

(16) Bacilio-Jiménez, M.; Aguilar, S.; Velásquez, M.; Pérez, A.; Zepeda, A.; Zenteno, E. Endophytic bacteria in rice seeds inhibit early colonization of roots by *Azospirillum brasilense*. *Soil Biol. Biochem.* **2001**, *33*, 167−172.

(17) Martínez, M. A.; Zenteno, E.; Cordoba, F. Partial purification and characterization of teosinte coleoptile. *Lectins, Biology, Biochemistry, Clinical Chemistry*; Bog-Hansen, T. C., van Driessche, E., Franz, H., Eds.; TEXTOP: Hellerup, Denmark, 1985; Vol. 8, pp 132−136.

(18) Bradford, M. M. A rapid and sensitive method for quantitation of microgram quantities of proteins utilizing the principle of protein dye-binding. *Anal. Biochem.* **1976**, *37*, 248−254.

(19) Hedrick, J. L.; Smith, A. J. Size and charge isomer separation and estimation of molecular weights of proteins by disc gel electrophoresis. *Arch. Biochem. Biophys.* **1968**, *126*, 155−164.

(20) Reisfeld, R. A.; Lewis, U. J.; Williams, D. E. Disk electrophoresis of basic proteins and peptides on polyacrylamide gels. *Nature* **1962**, *195*, 281−283.

(21) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

(22) Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L. Rapid analysis of amino acids using pre-column derivatization. *J. Chromatogr.* **1984**, *33*, 93−104.

(23) Hellman, U.; Wernstedt, C.; Gonez, J.; Heldin, C. H. Improvement of an "In-Gel" digestion procedure for the micropreparation of internal protein fragments for amino acid sequencing. *Anal. Biochem.* **1995**, *224*, 451−455.

(24) Spiro, R. G.; Bhoyroo, V. D. Structure of the O-glycosidically linked carbohydrate units of fetuin. *J. Biol. Chem.* **1974**, *249*, 704−711.

(25) Spik, G.; Coddeville, B.; Montreuil, J. Comparative study of the primary structures of sero-, lacto- and ovotransferrin glycans from different species. *Biochimie* **1988**, *70*, 1459−1469.

(26) Zanetta, J. P.; Timmerman, P.; Leroy, Y. Gas−liquid chromatography of the heptafluorobutyrate derivatives of O-methylglycosides on capillary columns: a method for the quantitative determination of monosaccharide composition of glycoproteins and glycolipids. *Glycobiology* **1999**, *9*, 255−266.

(27) Zenteno, E.; Vazquez, L.; Chavez, R.; Cordoba, F.; Wieruszeski, J. M.; Montreuil, J.; Debray, H. Specificity of the isolectins from the plant cactus *Machaerocereus eruca* for oligosaccharides from porcine stomach mucin. *Glycoconjugate J.* **1995**, *12*, 699−706.

(28) Carrari, F.; Perez-Flores, L.; Lijavetzky, D.; Enciso, S.; Sanchez, R.; Benech-Arnold, R.; Lusem, N. Cloning and expression of a sorghum gene with homology to maize vp1. Its potential involvement in pre-harvest sprouting resistance. *Plant Mol. Biol.* **2001**, *45*, 631−640.

(29) Hernández, P.; Debray, H.; Jaekel, H.; Garfias, Y.; Jimenez, M. C.; Martínez-Cairo, S.; Zenteno, E. Chemical characterization of the lectin from *Amaranthus leucocarpus syn. hypocondriacus* by 2-D proteome analisis. *Glycoconjugate J.* **2001**, *18*, 321−329.

(30) Zenteno, E.; Ochoa, J. L.; Montaño, L. F.; Debray, H.; Montreuil, J. *Machaerocereus eruca* cactus isolectins: Purification and characterization. *Plant Sci.* **1991**, *77*, 11−19.

(31) Hill, H. D., Jr.; Schwyzer, M.; Steinman, H. M.; Hill, R. L. Ovine submaxillary mucin. Primary structure and peptide substrates of UDP-*N*-acetylgal-*N*-acetyllactosamine: mucin transferase. *J. Biol. Chem.* **1977**, *252*, 3799−3804.

(32) Schauer, R.; Casals-Stenzel, J.; Corfield, A. P.; Veh, R. W. Subcellular site of the biosynthesis of O-acetylated sialic acids in bovine submandibular gland. *Glycoconjugate J.* **1988**, *5*, 257−270.

(33) Schmid, K.; Binette, J. P.; Dorland, L.; Vliegenthart, J. F.; Fournet, B.; Montreuil, J. The primary structure of the asialocarbohydrate units of the first glycosylation site of human plasma α1-acid glycoprotein. *Biochim. Biophys. Acta* **1979**, *581*, 356−359.

(34) Egge, H.; Peter-Katalinic, J.; Paz-Parente, J.; Strecker, G.; Montreuil, J.; Fournet, B. Carbohydrate structures of hen ovomucoid. A mass spectrometric analysis. *FEBS Lett.* **1983**, *156*, 357−362.

(35) Chaumont, F.; Barrieu, F.; Wojcik, E.; Chrispeels, M. J.; Jung, R. Aquaporins constitute a large and highly divergent protein family in maize1. *Plant Physiol.* **2001**, *125*, 1206−1215.

(36) Rini, M. Lectin structure. *Annu. Rev. Biomol. Struct.* **1995**, *24*, 551−577.

(37) Kubanek, J.; Entlicher, G.; Kocourek, J. Studies on lectins. LII. Isolation and characterization of the lectin from rye germ (*Secale cereale L.*). *Acta Biol. Med. Ger.* **1982**, *41*, 771−780.

(38) Monsigny, M.; Roche, A. C.; Sene, C.; Maget-Dana, R.; Delmotte, F. Sugar−lectin interactions: how does wheat-germ agglutinin bind sialoglycoconjugates? *Eur. J. Biochem.* **1980**, *104*, 147−153.
(39) Tabary, F.; Balandreau, J.; Bourrillon, R. Purification of the rice embryo lectin and its binding to nitrogen-fixing bacteria from the rhizosphere of rice. *Biochem. Biophys. Res. Commun.* **1984**, *19*, 549−555.
(40) Matsuoka, Y.; Vigouroux, Y.; Goodman, M. M.; Sanchez, G. J.; Buckler, E.; Doebley, J. A single domestication for maize shown by multilocus microsatellite genotyping. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6080−6084.

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