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Studies of Storage Proteins of Higher Plants

I. CONCANAVALIN A FROM THREE SPECIES OF THE GENUS *CANAVALIA*[1, 2]

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ABSTRACT

Concanavalin A, the lectin of the Jack bean, *Canavalia ensiformis*, was extracted and compared with homologous proteins from *Canavalia gladiata* and *Canavalia maritima*. All proteins were bound to Sephadex G-100 and eluted from the gel with buffered glucose solution. Quantitative recoveries indicated that large quantities (23 to 28% of dry seed protein) of these lectins are synthesized by all three species. Antibody preparations made against *C. ensiformis* lectin failed to discriminate among the three proteins; the pattern of the precipitin bands indicated identical antigenic determinants in the Ouchterlony double-diffusion assay. Native and sodium dodecyl sulfate polyacrylamide gel electrophoresis also failed to distinguish differences in the proteins. The storage protein active in carbohydrate binding is composed, in each case, of identical subunits. However, the amino acid composition of the subunit chains from the three sources is not identical. In particular, the lectins from *C. ensiformis* and *C. gladiata* contain two methionine residues per protein subunit, while only one methionine residue is found in the *C. martima* lectin. Cyanogen bromide cleavage of the purified subunit from *C. maritima* yieded two fragments with molecular weights estimated at 20,400 and 4,600, respectively. Amino acid analysis of the separated fragments indicated that the methionine residue at position 130 in *C. ensiformis* is absent in the lectin from *C. maritima*.

During seed germination storage proteins are hydrolyzed, and the amino acids are transported into the growing seedling axis. However, certain storage proteins, called lectins and found largely in the cotyledons of legumes, have the capacity to bind specific carbohydrates. The functional significance of this property is unknown, but there have been several reports indicating that lectins have an affinity for the cell surface of species of *Rhizobium* and may, therefore, facilitate the entry of these symbiotic, nitrogen-fixing organisms into the root cortex (8, 18).

Relatively little is known about the mechanism of higher plant protein synthesis in comparison to that of microorganisms and animal tissues (38). A cell-free system from higher plant tissues that synthesizes a characterizable plant protein would be a useful tool in unraveling the complexities of the general synthetic process. Work carried out by Boulter and his associates (6, 7, 9, 26, 37) and by Millerd, *et al.* (21) on synthesis of legumin, a storage protein of *Vicia faba*, indicates that developing seeds may eventually provide such a system. Legumin is synthesized at a rapid rate in the cotyledons as cell division ceases and over the course of cell expansion the quantity increases from zero to about 30% of the total protein of the seeds (21). The stimulus for initiation of storage protein synthesis, the mechanism of synthesis on the rough endoplasmic reticulum, and the deposition of this protein in membrane-bound sacs are special aspects of storage protein synthesis also requiring further investigation.

Developing seeds of *Canavalia ensiformis* and their storage protein, Con A,[3] have several characteristics that make this an attractive system for study. Immunologists have been interested in the action of Con A on animal cells since its identification as the hemagglutinin of the Jack bean by Sumner and Howell in 1935 (31). This lectin has an affinity for certain carbohydrates and binds to sites on the plasmalemma of animal cells (10, 29) and plant protoplasts (16) bearing receptor sugars.

The strong affinity of Con A for branched polysaccharides with terminal nonreducing glucopyranosyl residues causes it to bind to dextran gels. After the gel is washed to remove other proteins, Con A may be eluted in a highly purified state by adding glucose, a competitive inhibitor of the Con A-dextran interaction, to the elution buffer (2). The purified lectin contains no carbohydrate, unlike the glycoproteins isolated from several other legumes (29).

Structural studies of Con A have accompanied those of carbohydrate-binding activity and have led to the recent publication of the complete primary sequence and the three-dimensional structure of this protein molecule (13, 19).

The ease of purification, the fact that the complete structure of Con A from *C. ensiformis* is known, and the possibility (by analogy with other legumes) that this storage protein is rapidly synthesized during a particular developmental stage led me to favor *Canavalia* seed material for my initial studies of storage proteins. I have investigated the quantity of dextran-binding protein present in dry seeds of three species of Canavalia, *C. ensiformis*, *C. gladiata*, and *C. maritima*, looking for species that lay down large quantities of the storage protein and which would therefore be useful for subsequent work on the mechanism of protein synthesis. I also compared purified proteins of these same species with respect to their reaction to a single antiserum, their electrophoretic properties, and their amino acid composition, seeking possible

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[2] Portions of this work were presented at the annual meeting of the American Institute of Biological Sciences, Minneapolis, Minn. in August, 1972.

[3] Abbreviations: Con A: Concanavalin A; SDS: sodium dodecyl sulfate.

differences in the structure of this protein among the three species.

MATERIALS AND METHODS

Protein Preparation. Concanavalin A was extracted from small samples (usually 0.5 g) of finely milled seeds prepared by removing seed coats from five to six seeds and grinding with mortar and pestle. Samples were extracted three times over an 18-hr period at 4 C with a total of 35 ml of extraction medium (1 M NaCl, 10 mM tris-Cl (pH 7.4), 1 mM $MgCl_2$, and 1 mM $CaCl_2$). Insoluble material was pelleted at 30,000*g* after each extraction. The pooled supernatant fluid was applied slowly to a G-100 Sephadex (Pharmacia Fine Chemicals, Inc.) column (2.2 × 11 cm), and the gel was washed with 30 mM NaCl containing tris-Cl, $MgCl_2$, and $CaCl_2$ as above. When the absorbance at 280 nm dropped below 0.01, glucose was added to the eluting buffer (10 g/100 ml). A single peak of 280-nm absorbing material was eluted with the glucose solution (2). When further purification was needed, the glucose-eluted protein solution was dialyzed and reapplied to a G-100 column for a second elution with glucose. Bound carbohydrate was removed completely from the lectin by making the glucose-protein solution from the G-100 column 1 M in acetic acid, stirring for 20 min, and dialyzing against column buffer solution and subsequently against distilled H_2O (23).

Quantitative Determinations of Protein. Total soluble protein in the seed extract was determined by the biuret method as modified by Munkers and Richards (22). Portions of the extract were precipitated with 10% trichloroacetic acid, washed with ethanol, dried, and dissolved in 1 M NaOH for biuret analysis. The quantity of purified Con A was estimated from the absorbance at 280 nm, $E^{1\%}_{1\,cm} = 11.4$ (3), as well as biuret analysis. Values for total protein were checked by Microkjeldahl nitrogen determination, and the quantity of nitrogen in the residual pellet after extraction was also determined by this technique.

Immunodiffusion Studies. Immunodiffusion studies were carried out by the Ouchterlony method (24) as modified by Crowle (11). Antisera were purchased from Miles Laboratories, Inc., Elkhart, Ind. The serum was prepared in goats challenged with Con A extracted from *C. ensiformis*. Equivalence concentrations of antigen and antibody were estimated by precipitation in capillary tubes. Equal volumes (5 μl) of these concentrations (1:1 sterile H_2O dilution of goat antiserum and 0.5 mg/ml antigen protein) were applied to appropriate wells cut in an agarose medium poured on glass slides. The agarose (0.7%) was dissolved in 0.1 mM acetate buffer (pH 5.2) containing 0.15 M NaCl. Antigen proteins were dissolved in the same buffer containing 5% α methyl mannoside, and diffusion was allowed to proceed overnight at 37 C.

Polyacrylamide Gel Electrophoresis. Discontinuous gel electrophoresis at pH 4.5 with 7.5% acrylamide gels was carried out according to the method of Reisfield *et al.* (27). Basic fuchsin was used as a marker dye and 6 mamp current was applied to each tube.

SDS gels were prepared by the method of Weber and Osborn (36). Protein preparations were heated at 80 C for 10 min in the presence of 2.5% SDS before applying them to the 10% gels. Electrophoresis was carried out at 6 mamp per tube for 6 hr. Estimation of molecular weights of subunits and subunit fragments were made in 10 and 15% SDS gels using Cyt *c*, mol wt 11,700; pancreatic ribonuclease, 13,700; carbonic anhydrase, 29,000; and ovalbumin, 43,000, as standards.

All gels were stained with Coomassie blue and destained by diffusion.

Amino Acid Analysis. Protein samples were hydrolyzed at 110 C in 6 N HCL *in vacuo* for 24, 48, or 70 hr. Amino acid analyses were made with a Spinco Model 180 analyzer. Tryptophan values were not determined.

Preparation of Subunits of Concanavalin A. Large quantities of purified lectins were prepared by scaling up methods explained above. Intact subunit chains were prepared by: (*a*) incubation of purified protein (10 mg/ml) in 1% NH_4HCO_3 at 31 C for 16 hr followed by centrifugation and dialysis of the supernatant solution containing intact subunits (12) or (*b*) dissociation of the purified protein in 8 M deionized urea and subsequent separation of intact subunits and natural fragments of subunits on a column of Sephadex G-100 (120 × 5cm) on 8 M urea and 0.05 M glycine-Cl buffer, pH 3 (1).

Cyanogen Bromide Cleavage of Subunit Protein. Cyanogen bromide was used to cleave peptide chains at internal methionine residues (17). Subunits were cleaved with an excess of cyanogen bromide dissolved in 70% formic acid (34). The reaction was allowed to proceed for 5 hr at room temperature; longer reaction times gave similar results. To terminate the reaction, a 15-fold volume of distilled H_2O was added to the reaction flask, and the mixture was frozen in Dry Ice and acetone and lyophylized.

Separation of Cyanogen Bromide Cleavage Products. The lyophylized peptide mixture was dissolved in 4 M guanidine chloride, heated to 75 C for 10 min, and applied to a 2 cm × 90 cm column of Bio-Gel A-5m (Bio-Rad Laboratories) for elution with the same solvent. The gel column was previously calibrated with a series of purified proteins from 60,000 to 3500 in mol wt, and a standard plot was made of the logarithm of molecular weight against the relative elution position. Molecular weights of the unknown cyanogen bromide fragments were estimated on this curve (15).

RESULTS

The elution profiles shown in Figure 1 are typical of the separation of the dextran-binding protein on Sephadex G-100. If the A_{280} absorbing material that does not bind to the dextran is reapplied to fresh gel, it is completely eluted with buffer in the absence of glucose. Thus, quantitative recovery of the dextran-binding protein is possible in a single chromatographic run.

To test the efficacy of this procedure in purification of dextran-binding proteins, the glucose-eluted protein solution was dialyzed and reapplied to a buffer-washed column. There was no measurable absorbance at 280 nm in the buffer wash, and 95% of the protein applied was recovered on glucose elution.

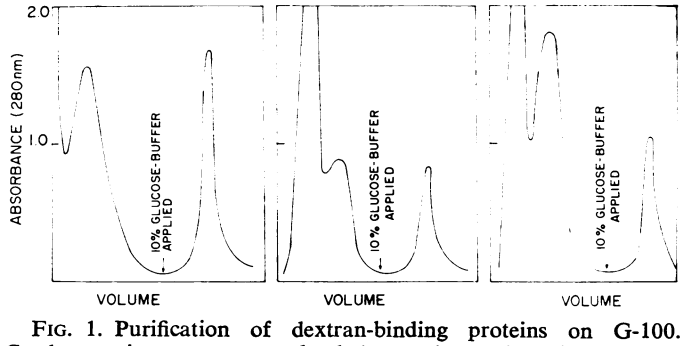

FIG. 1. Purification of dextran-binding proteins on G-100. Crude protein extracts were loaded on columns in Tris-Cl buffer, pH 7.4. Vertical arrow indicates addition of 10% glucose (w/v) to the eluting buffer. A: *C. maritima;* B: *C. ensiformis;* C: *C. gladiata.*

Since Con A binds to the membranes of certain higher plant protoplasts (16), it seemed reasonable that a fraction of the protein might be bound to insoluble material during the seed extraction procedure. Therefore, a sample of seed material was extracted with buffer solution containing 10% glucose. After dialysis to remove the sugar, this extract was applied to a G-100 column. No increase in the yield of the lectin was observed.

Quantitative data on protein fractions are given in Table I. These data were obtained from a single lot of seeds. One seed lot of *C. gladiata,* two lots of *C. ensiformis,* and several lots of *C. maritima* have been analyzed. *C. maritima* usually contained the largest quantity of dextran-binding protein. Among samples of *C. maritima* themselves, two from Little Cayman Island contained the largest quantity of lectin (5.5 and 5.4% of the dry weight, respectively) while samples from Jamaica and Trinidad contained somewhat less (4.5–5.0%). In all cases the dextran-bound protein constituted a large fraction of the total protein. This fraction ranged from a low of 10% for one sample of *C. ensiformis* (unable to repeat analysis because of lack of material from this seed lot) to a high of 37% for one sample of *C. maritima* protein.

The quantity of lectin in all three species examined is such that the developing seed system of any one could be used to study protein synthesis. Concanavalin A from *C. ensiformis,* however, is the only dextran-absorbed protein from plants of the genus *Canavalia* that has been studied. Therefore, I decided to compare, qualitatively, the protein extracted from *C. ensiformis* with those showing the same binding specificity extracted from *C. gladiata* and *C. maritima,* respectively.

Table I. *Determination of Protein in Dry Seed Meal*

Data are from one seed lot of each of the three varieties analyzed simultaneously. Data in parentheses represent range of extremes found on analysis of several seed lots.

	Total Protein	Dextran-bound Protein		
	mg/g tissue	*mg/g tissue*	*% total protein*	*% dry wt*
C. gladiata	141	39	28	3.9
C. maritima	175	55	32	5.5 (4.5–6.3)
C. ensiformis	154	35	23	3.5 (2.0–3.5)

Figure 2A illustrates the reaction of Con A antiserum with purified protein preparations from the three sources. The precipitin bands formed by reaction of the antigens with the common antiserum are fused and no spurs are observed. This result is the "reaction of complete identity" and indicates that the three proteins have common antigenic determinants. Figure 2B illustrates a control preparation in which normal goat serum was applied in the center well. No precipitin reaction occurs under these conditions.

In order to avoid the formation of spurious precipitin lines, it was essential to apply the antigen proteins in solution containing 5% α methyl mannoside. This is the monosaccharide

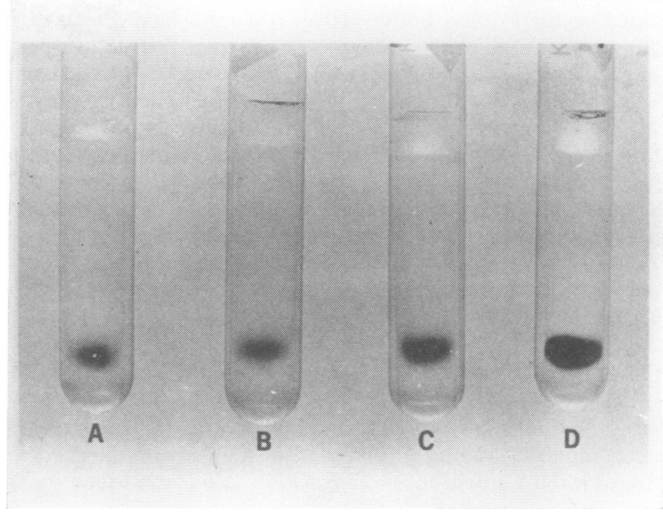

FIG. 3. Disc electrophoresis of dextran-purified proteins from seeds of A: *C. gladiata;* B: *C. maritima;* and C: *C. ensiformis.* D contained a mixture of proteins from all three sources.

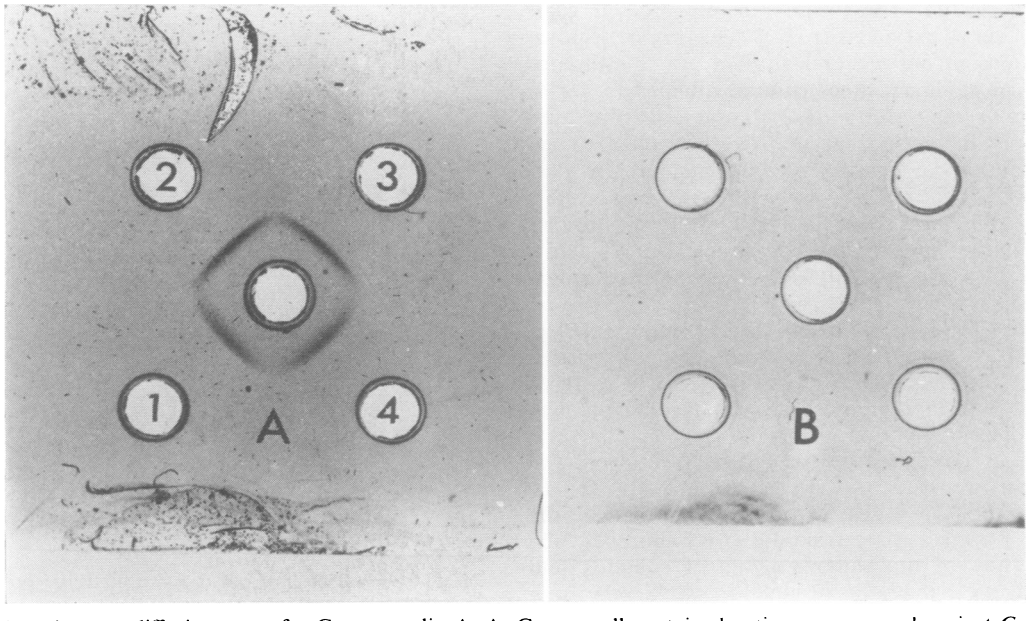

FIG. 2. Ouchterlony immunodiffusion assay for Concanavalin A. A: Center well contained antiserum prepared against *C. ensiformis* Con A; 1, 2, and 3 contained dextran-purified proteins from *C. maritima, C. ensiformis,* and *C. gladiata,* respectively; 4 contained a mixture of all three proteins. B: Same as A except normal serum in center well.

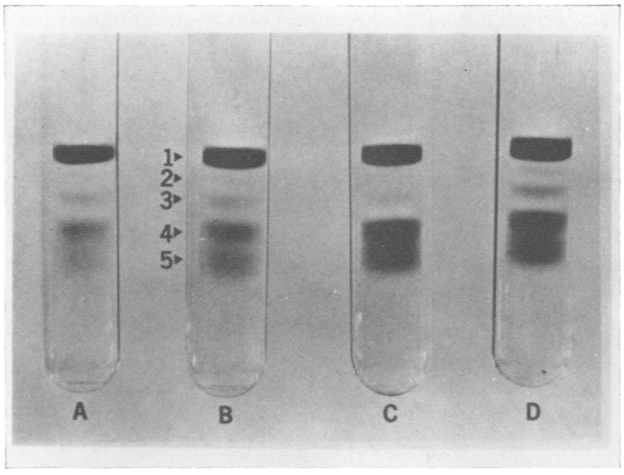

FIG. 4. SDS gel electrophoresis of dextran-purified proteins from seeds of A: *C. gladiata;* B: *C. maritima;* C: *C. ensiformis.* D contained a mixture of proteins from all three sources. Band 1 represents the intact subunit and bands 2 to 5 represent natural fragments of this peptide chain.

Table II. *Amino Acid Composition of Concanavalin A*

Data are expressed as moles amino acid per 25,500 g protein, the subunit weight of Con A from *C. ensiformis.*

	C. ensiformis[1]	*C. ensiformis*[2]	*C. gladiata*[2]	*C. maritima*
Lys	12	12.27 (12)	12.14 (12)	11.62 (12)
His	6	6.33 (6)	6.36 (6)	6.02 (6)
Arg	6	5.63 (6)	5.42 (5)	5.77 (6)
Trp	4	ND[3]	ND	ND
Asp	33	34.37 (34)	35.54 (36)	33.75 (34)
Thr[4]	19	18.76 (19)	18.88 (19)	19.03 (19)
Ser[4]	31	30.32 (30)	27.81 (28)	29.81 (30)
Glu	13	11.81 (12)	11.23 (11)	11.16 (11)
Pro	11	11.46 (11)	11.41 (11)	9.85 (10)
Gly	16	16.22 (16)	17.97 (18)	19.23 (19)
Ala	18	18.15 (18)	17.21 (17)	17.56 (18)
Cys	0	0	0	0
Val[5]	17	15.51 (16)	15.89 (16)	17.66 (18)
Met	2	1.94 (2)	1.88 (2)	1.01 (1)
Ile	15	13.40 (13)	13.28 (13)	14.01 (14)
Leu	18	18.59 (19)	19.06 (19)	19.40 (19)
Tyr	7	7.04 (7)	7.30 (7)	7.12 (7)
Phe	10	10.90 (11)	11.03 (11)	10.77 (11)

[1] Edelman *et al.* (13).
[2] Single 24-hr hydrolysis.
[3] Not determined.
[4] Values extrapolated to zero time.
[5] 70-hr hydrolysis.

that binds most strongly to Con A (30) and may, therefore, inhibit binding of Con A to glycoproteins of the antiserums. It was also necessary to exclude CA^{2+} and Mg^{2+} from the antigen protein solution. Normally these two ions were included in the buffer solutions, for Con A is a metalloprotein binding one ion of Mn^{2+} or Mg^{2+} and one ion of Ca^{2+} per protein subunit (13). However, inclusion of the ions in the protein solution caused the appearance of nonspecific precipitin lines in the Ouchterlony assay.

The three proteins were also compared by polyacrylamide gel electrophoresis. Discontinuous gel electrophoresis of the proteins at pH 4.5 is illustrated in Figure 3. In each case the proteins ran as a single, noncompact, band near the marker dye.

When the proteins are dissociated with SDS, the gel pattern shown in Figure 4 results. There are no obvious differences in the banding patterns of the different proteins. The common pattern is similar to that found by Wang *et al.* (33) for Con A subjected to electrophoresis under the same conditions. Biologically active Con A is composed of two or four identical subunits; there is at least one labile peptide linkage in the monomer subunit. This bond is hydrolyzed in a fraction of the population of molecules under all extraction conditions tested (33). Nevertheless, cleaved subunits do participate in tetramer formation and carbohydrate binding. Only on SDS or urea treatment do the altered monomers break apart into fragments. By analogy with the results of Wang *et al.*, band 1 (Fig. 4) represents the intact subunit with mol wt of 25,800, band 3, a fragment of 18,000 mol wt not further analyzed by Wang's group, and bands 4 and 5 peptide fragments of mol wt 13,000 and 10,000, respectively. In addition to these previously identified bands, a faint band identified as 2 in Figure 4 also occurred in some gel preparations.

Amino acid composition data are presented in Table II. There is good proportional agreement between this analysis of *C. ensiformis* Con A and the composition data from the crystallographic analysis (13). The composition of Con A extracted from *C. gladiata* is similar to that of *C. ensiformis* and the significance of the minor differences between it and that of *C. ensiformis* is uncertain. Similar results were reported by Akedo *et al.* (4) for the *C. gladiata* lectin while this study was in progress. However, there is an interesting difference between the composition of *C. maritima* protein and that extracted from other sources. Con A from *C. maritima* contains only one methionine residue per subunit, half the number occurring in the other species. This fact is significant for one of the methionine residues in the *C. ensiformis* protein appears to be involved in subunit association (13, 19).

To determine the position of the methionine residue in the *C. maritima* subunit, the intact subunit was purified and subjected to cyanogen bromide. The cleavage products were subsequently separated on acrylamide gels. In attempting to purify this subunit, I first used a method of incubation in 1% NH_4HCO_3 (12). This treatment has been used in purification of Con A subunits from *C. ensiformis.* It causes broken fragments to precipitate, while intact units remain in solution. Control incubation with *C. ensiformis* protein gave the expected results, but it was not possible to purify the *C. maritima* lectin by this procedure (Fig. 5). Greater than 90% of the *C. maritima* protein precipitated during the incubation or on subsequent dialysis, and the soluble material after dialysis and centrifugation remained impure. The recovery of purified subunit from *C. ensiformis,* on the other hand, was 55% of the starting material.

C. maritima lectin was purified by chromatography on G-100 in the presence of 8 M urea (Fig. 6). Peak 2 contained material highly enriched (90%) in the intact subunit. After dialysis 1 mM $MgCl_2$ and lyophylization, this protein was cleaved with cyanogen bromide. Figure 7 illustrates SDS polyacrylamide gel electrophoresis of the subunit and cyanogen bromide cleavage products from *C. maritima* and *C. ensiformis.* In both cases the intact subunit has a mol wt estimated at 27,000 by gel chromatography. (The true mol wt of the *C. ensiformis* subunit is 25,500 [13].) The main cleavage product of the *C. maritima* protein has a mol wt estimated at 18,500 while the other, lighter staining, and more diffuse band represents material beyond the resolution of the gel. In the case of *C. ensiformis,* nearly all the material is cleaved to products

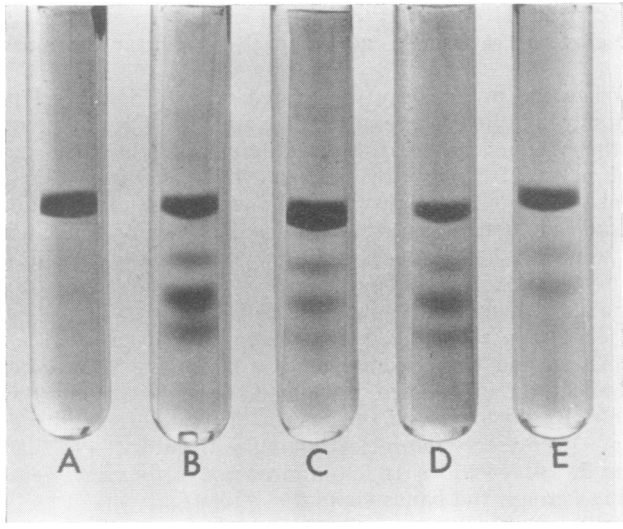

FIG. 5. SDS gels from purification of *C. ensiformis* (A and B) and *C. maritima* (C, D, and E) lectin subunits by 1% NH_4HCO_3 incubation at 37 C. A: Purified subunit after incubation and dialysis to remove NH_4HCO_3. Precipitation did not occur during dialysis. B: Fragment and subunit mixture precipitating during NH_4HCO_3 incubation. C: Mixture of subunits and fragments after NH_4HCO_3 incubation and dialysis. D: Fragments and subunits precipitating during incubation. E: Fragments and subunits precipitating during dialysis.

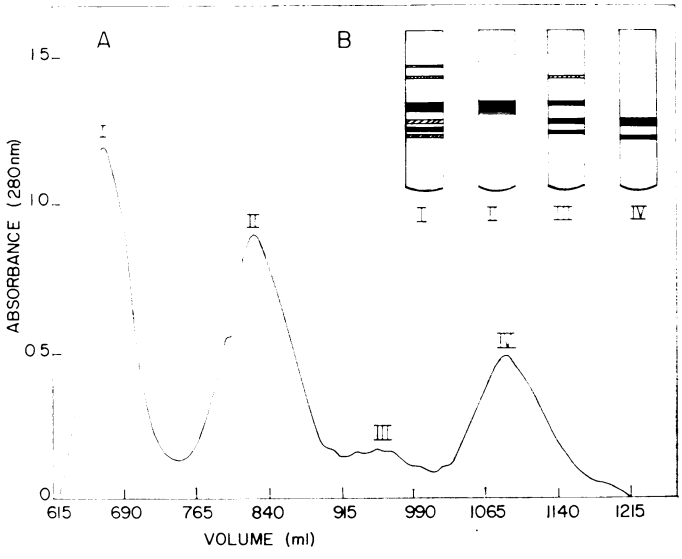

FIG. 6. A: Pattern of purified lectin from *C. maritima* dissociated with 8 M urea and chromatographed on Sephadex G-100 in 0.05 M glycine-Cl buffer, pH 3, containing 8 M urea. $275A_{280}$ units were loaded on column (5 × 120 cm) and eluted at a rate of 2 ml/min. B: SDS gel pattern resulting from electrophoresis of four main peaks resolved on G-100 chromatography. Peak II material contained the intact subunit and was relatively free of contaminating fragments.

beyond the resolving capacity of the gel. This latter result is in agreement with the data of Waxdal *et al.* (35) who determined mol wt of 4,700, 9,100 and 10,700 for cleavage products of *C. ensiformis* subunit by high speed equilibrium centrifugation. Similar gel profiles were obtained with the cleaved *C. gladiata* lectin subunit (not shown).

Finally, the cyanogen bromide cleavage products were separated on Bio-Gel A in 4 M guanidine chloride (Fig. 8). Molecular weights of 20,400 and 4,600 were estimated for the material eluting in peaks 1 and 2, respectively. Amino acid analysis of these fractions revealed a composition approximating very closely the analogous fragments of *C. ensiformis* Con A (Table IV and Fig. 9). Phenylalanine is absent from the F_1 fragment of *C. ensiformis* as well as the small fragment of the *C. maritima* protein. The sum of the mole fractions of amino acids of the *C. maritima* fragments is in close agreement with the mole fractions of amino acid in the native Con A molecule indicating that the intact subunit is simply the sum of the two cyanogen bromide fragments (Tables II and III).

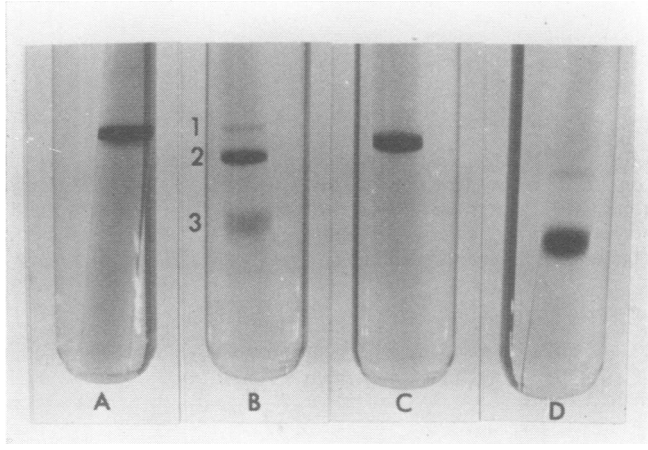

FIG. 7. SDS gels (12%) of 50 μg quantities of cyanogen bromide cleavage products of intact subunits of *C. maritima* lectin (A and B) and *C. ensiformis* lectin (C and D). A: Intact subunit prepared by 8 M urea chromatography; B: cyanogen bromide cleavage products; band 1: material of same mobility as intact subunits; band 2: large cleavage product with estimated mol wt of 18,500; band 3: lower mol wt material (<12,000) resulting from cleavage; C: Intact subunit prepared by NH_4HCO_3 incubation; D: cyanogen bromide cleavage products. Upper light band is in same relative position as band 2 in B and may represent those molecules cleaved only at position 40. The lower band contains the bulk of the fragments resulting from cyanogen bromide treatment (28).

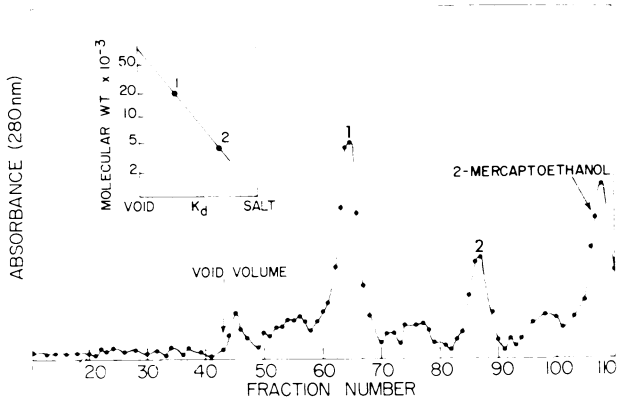

FIG. 8. Separation of products of cyanogen bromide cleavage of *C. maritima* lectin on Bio-Gel A. Void volume was determined with dextran blue and 2-mercaptoethanol was used to determine total volume of 4 M guanidine chloride in column. Inset shows position of peaks 1 (mol wt 20,400) and 2 (mol wt 4,600) on experimentally determined plot of log of mol wt × K_d.

$$K_d = V_e - V_o / V_i - V_o$$

V_e = elution volume for particular protein; V_o = void volume of gel; V_i = volume of solvent within and without gel matrix.

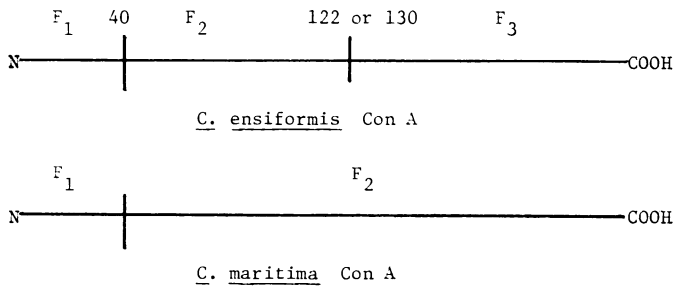

FIG. 9. Cyanogen bromide fragments of Con A. *C. ensiformis* data from Waxdal *et al.* (35) with methionine positions from data of Edelman *et al.* (13) and Hardman and Ainsworth (19). Relative position of cleavage of *C. maritima* Con A based on gel exclusion chromatography and similarity of amino acid analysis of F_1 to F_1 of *C. ensiformis* and F_2 to ($F_2 + F_3$) of *C. ensiformis*.

Table III. *Amino Acid Composition of Cyanogen Bromide Fragments*

C. ensiformis data are from Waxdal *et al.* (35). *C. maritima* data are based on F_1 fragment of 4,600 mol wt and F_2 fragment of 20,400 mol wt with same mole fraction tryptophan as *C. ensiformis*.

	C. ensiformis		*C. maritima*	
	F_1	$F_2 + F_3$	F_1	F_2
	moles amino acid/mole peptide			
Lys	3.8	9.5	3.91	8.51
His	1.1	5.3	1.31	5.17
Arg	0.9	5.3	1.25	4.49
Trp	1	4	ND[1]	ND
Asp	7.5	25.7	7.52	24.45
Thr[2]	4.0	14.4	3.00	13.28
Ser[2]	2.9	23.8	3.18	23.84
Glu	1.0	10.2	1.78	10.21
Pru	2.5	7.1	3.65	7.69
Gly	2.2	12.5	3.45	15.06
Ala	3.4	15.1	3.77	13.76
Cys	0	0	0	0
Val[3]	3.3	15.6	3.2	13.4
Hsr[4]	0.7	0.7	Present[5]	0
Met	0	0	0	0
Ile	4.9	9.1	4.62	8.72
Leu	0.9	16.4	1.20	15.87
Tyr	2.0	6.3	2.25	4.86
Phe	0	9.6	0	10.76

[1] Not determined.
[2] Values extrapolated to zero time.
[3] 70-hr hydrolysis.
[4] Homoserine + homoserine lactone.
[5] Not quantified.

DISCUSSION

The dextran-binding purification procedure, Ouchterlony assays and gel electrophoresis give evidence that the lectins from all three sources are similar in activity and structure. However, the amino acid analyses revealed a significant difference in composition of the *C. maritima* protein. This molecule has only one methionine residue per subunit while the *C. ensiformis* and *C. gladiata* lectins contain two molecules of this amino acid. According to x-ray crystallographic analysis of *C. ensiformis* Con A, the position of the methionine residues are 42 (11, 19) and 130 (11) or 122 (19). Cyanogen bromide cleavage, separation of the fragments and amino acid analysis are consistent with the conclusion that methionine at position 122 or 130 in Con A is absent from the *C. maritima* lectin.

X-ray crystallography results also indicate that the methionine residue near the middle of the Con A molecule is in the region of hydrogen bonding of identical subunits. It is possible, therefore, that substitution of one amino acid for another in this position will modify subunit interaction. Failure of the NH_4HCO_3 incubation technique to cause selective precipitation of subunit fragments of *C. maritima* Con A could be related to such a modification. However, until more is known about subunit interactions in this protein species, this inference will remain speculative.

Jonathan Sauer (28), a student of the genus *Canavalia*, suggests that *C. maritima*, a pan-tropical, littoral species, may be ancestral to inland species. Of the three species investigated here, only *C. maritima* synthesizes a lectin with one methionine residue per polypeptide chain. On the basis of this small sample, the conservative inference in keeping with Sauer's suggestion is that the two derived species (*C. ensiformis* and *C. gladiata)* have maintained the ancestral methionine composition while an evolutionary change has occurred in the genes coding for the lectin subunit in *C. maritima*, reducing the methionine residues from two to one. However, I should point out that *C. ensiformis* and *C. gladiata* are domesticated species and, therefore, have been subject to special selection pressures. Now that the structure of *C. ensiformis* Con A is known and the *C. maritima* variant has been partially described, information on this lectin in the other members of this genus of about 50 species (28) will be of use to those interested in the evolution of plant proteins.

Evolution has produced a group of plant proteins, the lectins, containing large quantities of aspartic acid, serine and threonine, and small amounts of the sulfur-containing amino acids. Concanavalin A is typical in this respect (29). Also, all those lectins investigated have been found to be metallo-proteins containing Mn^{2+} and Ca^{2+} and to require these metal ions for carbohydrate-binding activity (25, 29). The function of lectins in the plant is unknown, and (*a*) protection against fungi (5), (*b*) mediation of sugar storage and transport (14, 31), (*c*) control of cell division during germination (29), and (*d*) involvement in entry of *Rhizobium* species into the root cortex (8, 18) have been advanced as possibilities. There is no compelling evidence to support or reject these alternatives, or to rule out further possibilities. There is a great difference in the relative amounts of lectins in different seeds, for example 5.5% of dry seed weight of *C. maritima* is Con A, while the lectin of *Lens culinaris* accounts for only 0.02% of the dry seed weight and a small fraction of the total protein (20). Apparently, in some seeds the lectin, in addition to any other role it may play, has become the main storage protein of the seed, while in others the lectin is limited to a function requiring only small quanities of the protein.

The suggestion that lectins may be involved in cell division *in situ* is intriguing, and we are testing this hypothesis by incubating protoplasts of the Jack bean and other species with Con A.

The results show that large quantities of Con A are laid down by all three species investigated. The ease of purification and extensive knowledge of the structure of this lectin make this system a likely tool for the study of protein synthesis in higher plants. The rate of protein synthesis in the developing seed, the loss of protein from the cotyledons during germination, and the carbohydrate-binding specificity of the Con A from *C. maritima* will be the subjects of subsequent papers.

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