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Short Communication

Purification and Partial Characterization of Two Lectin Isoforms from *Cratylia mollis* Mart. (Camaratu Bean)

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ABSTRACT

Two additional electrophoretically distinct molecular forms, isoforms (iso) 2 and 3, with lectin properties were isolated from *Cratylia mollis* Mart. seeds (FABACEAE), by extraction with 0.15*M* NaCl and ammonium sulfate fractionation, followed by chromatography on Sephadex G-75 and Bio-Gel P-200 (iso 2), as well as CM-Cellulose and Sephadex G-75 (iso 3). Both isoforms were human group nonspecific and showed distinct specificity. Polyacrylamide gel electrophoresis resolved iso 2 and 3 in polypeptides of apparent mol wts 60 and 31 kDa, respectively; a distinct isoelectric focusing pattern was obtained for iso 2 and 3, under denaturing and reducing conditions.

Index Entries: Lectin; multiple molecular forms.

INTRODUCTION

Proteins of nonimmune origin, with the property of agglutinating cells by interacting through at least two carbohydrate binding sites (*1*) and presenting hydrophobic sites (*2–5*), are called lectins. Some species

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contain two or more proteins with hemagglutinating activity, e.g., *Sambucus nigra* (*6*), *Vicia villosa* (*7*), and *S. sieboldiana* (*8*). The multiple molecular forms of lectins present in extracts have been called isophytohemagglutinins (*9*) or isolectins (*10,11*) and they may have originated from different genes (*10*). The term isoform seems proper to designate multiple molecular forms present in the same species, variety, or cultivar of nondefined genetic origin. In seeds of *Cratylia mollis*, a native FABACEAE, of forage use "in natura" in the semiarid region of the northeast of Brazil, an isoform 1 (iso 1) of the lectin was initially purified by M. T. S. Correia and L. C. B. B. Coelho (unpublished data). In this report, we describe the isolation and partial characterization of two additional lectin isoforms (iso 2 and 3), present in minimal amounts in *C. mollis* seed extracts but of use in cell surface studies and contributing to the pool of species protein heterogeneity.

METHODS

Hemagglutinating Activity (HA)

Glutaraldehyde-treated erythrocytes were used (*12*). The hemagglutination titer was expressed as the lowest protein concentration of the preparation at which full agglutination of erythrocytes was observed.

Protein Evaluation

Whenever necessary, the protein was estimated according to Lowry et al. (*13*) and by absorbance at 280 nm.

Purification and Partial Characterization of Isoforms

C. mollis seed meal was extracted with 0.15*M* NaCl (10% w/v) at 4°C, for 16 h and solid ammonium sulfate was added to the crude extract. A 40% saturated precipitate dialyzed against distilled water, followed by 0.15*M* NaCl and 10 m*M* citrate phosphate buffer, pH 5.5 (F0-40) and a supernatant of a 40–60% saturated fraction dialyzed twice, first against distilled water, followed by 0.15*M* NaCl (SF40-60), were obtained. To purify iso 2 (room temperature, RT), SF40-60 was chromatographed on a Sephadex G-75 column (6.0×1.46 cm) and the unadsorbed fractions were passed through a Bio-Gel P-200 column (6.0×1.46 cm). To obtain iso 3, F0-40 was applied on a CM-Cellulose column (31.0×1.44 cm), at 4°C, and the proteins were eluted with a 300 mL linear gradient of NaCl (0–0.4*M*). The highest HA peak obtained was chromatographed at RT, on a Sephadex G-75 column (6.5×1.0 cm). A hemagglutinating inhibitory assay was performed with monosaccharide solutions in 0.15*M* NaCl (200.0–1.5 m*M* *N*-

Table 1
Summary of Iso 2 and 3 Purifications

Sample	Volume, mL (v)	Protein mg/mL (a)	Titer, (b)	Specific activity (b/a)	Total activity (b x v)	Yield (%)
ISO 2						
SF40-60	910	2.50	1024	410	931840	2.6*
Preparation	30	1.80	2048	1113	61440	60**
Purified	3	0.07	512	7314	1536	75**
ISO 3						
F0-40	71	18.20	16384	900	1163264	3.3*
Preparation	30	0.30	64	191	1920	4.5**
Purified	12	0.20	64	355	768	92.5**

†Titer was obtained by mixing a twofold serial dilution of a lectin sample (50 μL) in 0.15*M* NaCl with 50 μL of a 2.5% (v/v) suspension of rabbit erythrocytes, in microtiter plates.

*Percentage of total activity recovered from the crude extract.

**Total activity recovered × 100/total activity chromatographed.

acetylglucosamine, D(+)-galactose, D(+)-glucose, D(+)-fructose, D(+)-mannose, α-methyl-D-galactoside, β-methyl-D-galactoside, α-methyl-D-mannoside, α-D(+)-fucose, lactose, melibiose, and sucrose) and rabbit erythrocyte suspension (*14*). Polyacrylamide gel electrophoresis was performed according to Laemmli (*15*) and the gels were carbohydrate stained (*16,17*); isoelectric focusing was made according to O'Farrell (*18*).

RESULTS

ISO 2

An 89% recovering of unadsorbed protein with the HA was obtained by Sephadex G-75 chromatography (iso 2 preparation). This preparation was resolved into two peaks (I and II), in Bio-Gel P-200. Peak I (purified iso 2) contained 12% of the applied protein sample and a much higher specific activity (Table 1).

ISO 3

The elution profile of the CM-cellulose column revealed four main peaks (I, II, III, and IV). Peak IV (iso 3 preparation) gave the highest specific activity. When iso 3 preparation was applied to a Sephadex G-75 column, most of the protein did not bind (purified iso 3) and showed a much higher specific activity (Table 1).

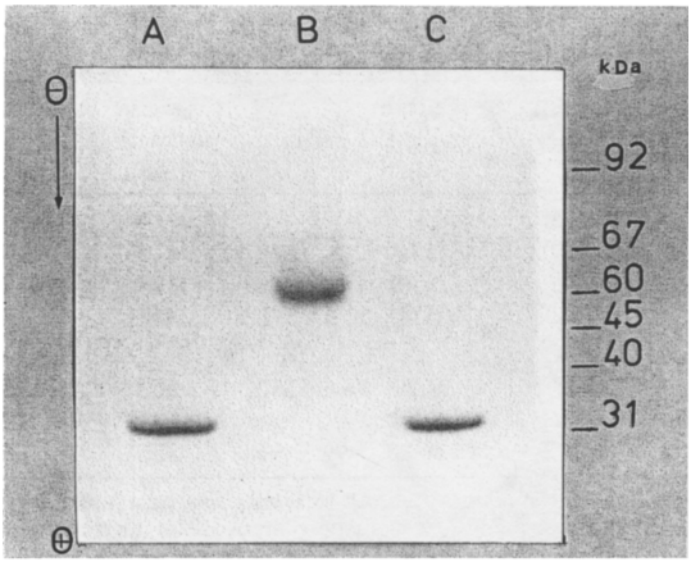

Fig. 1. SDS-polyacrylamide gel electrophoresis of the purified isoforms. Samples of iso 1 (A), 2 (B), and 3 (C), containing 25 μg of protein were reduced with β-mercaptoethanol and denatured by heating in the presence of SDS. Protein was stained with Coomassie brilliant blue according to Laemmli (*15*). Purified iso 1 was kindly supplied by M. T. S. Correia.

Iso 2 and 3 nonspecifically agglutinated human erythrocytes and the best monosaccharide inhibitors were α-methyl-mannoside (iso 2) and D(+)-galactose (iso 3). Iso 2 and 3 revealed polypeptides with apparent mol wts of 60 and 31 kDa, respectively (Fig. 1); a distinct isoelectric focusing pattern was obtained for iso 2 (pH range of 4.15–6.7) and 3 (pH range of 5.25–5.8). According to the carbohydrate staining used, iso 2 did not seem to be a glycoprotein. However, iso 3 was positive with Schiff's reagent and concanavalin A-peroxidase assay.

DISCUSSION

Through ammonium sulfate fractionation of *C. mollis* extract, a high recovery of iso 1 (94%) was obtained; however, there was evidence for other lectin molecular forms, which were present in F0-40 (3.3%) and SF40-60 (2.6%). Purified native isoforms 1, 2, and 3 had different electrophoretic migration (results not shown). Since the three isoforms were also detected in the extract, it excluded the possibility of artefactual modification products. Multiple forms have already been mentioned as a characteristic of plant lectins. It is possible that if exhaustively explored, lectin preparations would reveal heterogeneity of value, even if the active components are present in minimal proportions.

Isolectins have been described with the same or distinct erythrocyte agglutination or carbohydrate specificity (*19–21*). Most of the subunits of the legume lectins are composed of single polypeptide chains, however, there are some with two polypeptides (*10*). Iso 1 and 3 showed a subunit with the same apparent mol wt; these forms were resolved in polypeptides with distinct pI values. Differences have been detected in amino acid analysis (unpublished data) as well as in subunit components of iso 2 and 3. However, according to Moss (*22*), it is only through distinctions to a greater or lesser extent in their amino acid sequences that isoenzymes can be defined as such. Lectin molecular forms, purified from the same species, could be of value to molecular study of homologous proteins (*23*), to clarify subtle alterations in cell surfaces (*24–26*), and to structural evaluations, contributing to the supply of information available for speculation about functions in plant physiology.

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