BIOLOGIA PLANTARUM (PRAHA)

25 (5) : 343—348, 1983

Lectins from the Genus *Artocarpus*

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Abstract. The lectins from the seeds of two species of the genus *Artocarpus* (*A. integrifolia* L. and *A. incisa* L.) were isolated by affinity chromatography on an AB(+) human-stroma column, and compared by physicochemical and immunochemical methods. Although with marked differences in the hemagglutinating activity, the elution pattern of the albuminic and globulinic fractions from both seeds were comparable by affinity chromatography on a human-stroma column. The two isolectin families presented many similarities. All the lectins presented the same subunit pattern by SDS-polyacrylamide gel electrophoresis, were recognised by both rabbit antisera prepared against the crude extracts from the two seeds, and presented fused rockets when electrophoresed in agarose gel containing one or the other antiserum.

The phylogenetic distance between two individuals can be measured by structural differences of some of their metabolically important compounds. The degree of preservation of a particular protein, through the evolutionary process, is a good measure of the importance of such substance for the survival of the species. Some lectins isolated from different sources present similarities in their primary and secondary structures, as well as in the carbohydrate-protein linkage (FORIERS *et al.* 1978, 1979). HANKINS *et al.* (1979) showed, on the other hand, the formation of cross reactions between lectins isolated from several leguminous seeds, revealing the preservation of the structure through the evolutionary process.

In a previous paper (MOREIRA and OLIVEIRA 1983) the authors compared the proteins in general and the lectins in particular, of the seeds from two *Artocarpus* species. The lectins presented a closer relationship than the other major proteins. In the present paper the lectins isolated by affinity chromatography on an AB(+) human-stroma column, from seeds of the two *Artocarpus* species (*A. integrifolia* and *A. incisa*) were compared using physicochemical and immunochemical methods.

Received September 14, 1982; accepted December 1, 1982

MATERIAL AND METHODS

Reagents

Agarose was supplied by Sigma Chemical Co., Sephadex G-25 was supplied by Pharmacia Fine Chemical Inc., acrylamide, and N, N'-methylenebisacrylamide were from Eastman Kodak Co.

Plant Material

Artocarpus incisa L. (var. *seminifera*) and *Artocarpus integrifolia* L. (var. *mole*) were obtained from a local market.

Erythrocyte Samples

AB(+) human erythrocyte samples were obtained from healthy donors in blood banks.

Hemagglutinating Activity

The hemagglutinating activity was determined using the methods described by MOREIRA and PERRONE (1977).

Extraction of Proteins

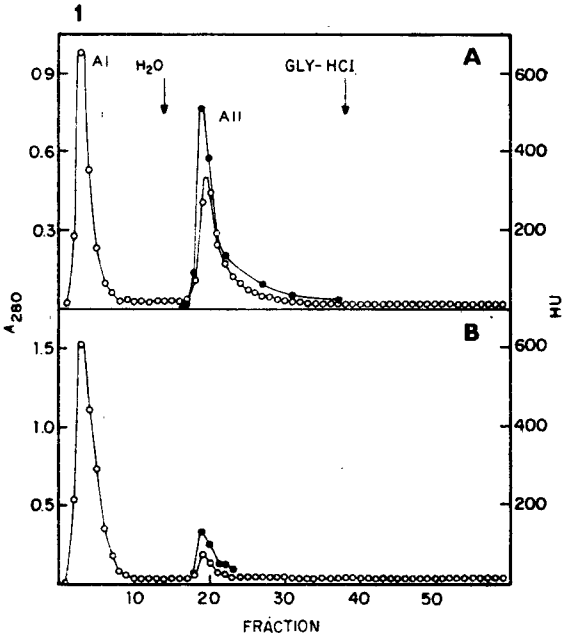
Crude extracts, albumins and globulins from *A. incisa* and *A. integrifolia* were prepared as described previously (MOREIRA and OLIVEIRA 1983).

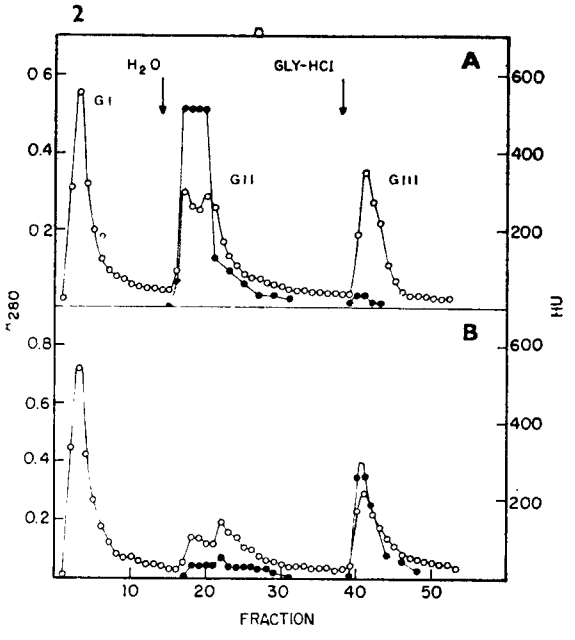
Immunization

Adult rabbits were immunized with intramuscular injection of the crude extracts (10 mg) emulsified in Freund's complete adjuvant. Further booster doses of the crude extracts (10 mg) in 0.15 M NaCl were injected to increase the antibody titer. The rabbits were bled by cutting the ear vein. The sera were extensively dialysed against water and freeze-dried.

Affinity Chromatography

Albumins and globulins (15 mg) were dissolved in 1.5 ml of phosphate buffered saline, PBS (0.025 M sodium phosphate buffer pH 7.4, containing 0.15 M NaCl) and applied to a human-affinity column (1.9×4.5 cm). The human-stroma were prepared by following the procedure of OCHOA and KRISTIANSEN (1978) modified for the use of human red blood cells. Glutaraldehyde treated stroma from AB(+) red blood cells were obtained by modification of the method of AVRAMEAS *et al.* (1974). The erythrocytes were washed three times with PBS and then lysed with distilled water (10 times the volume of packed cells). The mixture was then centrifuged at 15 000×*g* for 20 min at 4 °C and the precipitate washed exhaustively with 0.2% NaCl. To the loose sediment precipitated 2 M acetate buffer pH 5.0 (corresponding to 1/10 of the precipitate volume) was added, and glutaraldehyde in a precipitate + buffer to glutaraldehyde of 4 : 1. After standing for 3 h at room temperature, the insoluble derivative was washed extensively with PBS and incubated overnight in the freezer, with excess of 0.02 M glycine-HCl buffer (pH 2.6) in order to neutralize free aldehyde groups. The mixture was first washed with PBS, followed by extensive washing with water, homogenized in a Potter homogenizer and freeze dried. The affinity column was prepared with a mixture of stroma and swelled Sephadex G-25 superfine in a protein to dextran ratio of 1 : 10 (m/v). The column was washed with PBS, water and 0.02 M glycine-HCl buffer, until the absorbance





Figs. 1 and 2. Chromatography of *A. integrifolia* (A) and *A. incisa* (B) albumins on AB(+) human-stroma affinity column equilibrated with PBS. The elution was carried out first with the equilibration buffer followed by distilled water and then by 0.2 M glycine-HCl (pH 2.6) buffer. Fractions of 3.6 ml were collected at a flow rate of 24 ml h^{-1}. The figures show protein concentration (○) measured at 280 nm and hemagglutinating activity (●). — Fig. 1. Albumins. — Fig. 2. Globulins.

of the effluent fell below 0.05. This washing procedure was repeated before the application of each sample.

SDS-Polyacrylamide Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis was carried out with and without beta-mercaptoethanol in the protein buffer, following the method of WEBER and OSBORN (1969) as described by XAVIER-FILHO and MOREIRA (1978). Gels were stained with 0.005% (m/v) Coomassie Brilliant Blue in methanol-acetic acid-water (5 : 1 : 5 v/v/v).

Immunoelectrophoresis

This was carried out as described by CLAUSEN (1969) in agarose (1%) plates prepared with Veronal-lactate buffer pH 8.6. After an electrophoretic run the fractions were allowed to diffuse against the anti-crude extract sera for 24 to 48 h and the precipitated arcs examined by diffused light or by colouring the washed plates with Ponceau Red or Light Green.

Rocket Immunoelectrophoresis

These experiments were done in agarose (1%) gel containing antisera prepared against crude extracts (1. 27%), following the method described by WEEKE (1973). Precipitated arcs were observed as described above.

Isoelectric Focusing

This was done on 4% polyacrylamide gel slabs, at 7 °C, following the method described by PUSZTAI and STEWARD (1978). The final concentration of ampholines (pH 3 to 10) was 2.5%. The pI were determined by comparison with standard proteins.

RESULTS

Preparation of Lectins

Albumins and globulins of *A. incisa* and *A. integrifolia* were fractionated by affinity chromatography on a human-stroma column (Figs. 1 and 2). The albumins presented a single active peak and the globulin presented 2 peaks with activity. The chromatographic patterns were similar for both seeds.

SDS-Polyacrylamide Gel Electrophoresis

When the crude extract, the albumin and globulin fractions, and the active peaks were submitted to electrophoresis on polyacrylamide gel, it was found that the non-purified material presented several differences in subunit pattern (Figs. 3 and 4). The active peaks had the same subunit composition with bands at 11 500 and 15 000 dalton positions, the only exception being the glycine buffer eluted lectin from *A. integrifolia* globulins. This fraction presented a broad smear with a molecular mass running from 14 000 to 35 000 daltons when untreated and 6 000 to 12 000 daltons when treated with beta-mercaptoethanol, due probably to an inhibitor.

Immunoelectrophoresis

Crude extracts, albumins and globulins and active peaks of the two seeds were submitted to immunoelectrophoresis on agarose (1%) gel and the results obtained are shown in Figs. 5 and 6. Although the crude extracts,

albumins and globulins of the two seeds presented several bands when let to diffuse against the homologous antisera, only a few bands were recognized by the heterologous antisera. When the active peaks were submitted to the same experiment though, it was found that all the fractions were recognized by both antisera.

Rocket Immunoelectrophoresis

When the active peaks were submitted to electrophoresis on an agarose gel (1%) containing the homologous antisera (10%) several rockets were found for each peak (Fig. 7), indicating the presence of different proteins eluted in each peak. When these same peaks were electrophoresed together on agarose gel containing each of the two antisera (fused-rocket immunoelectrophoresis, Fig. 8) a complete fusion was found of all the rockets with the exception of a fast moving rocket present in the *A. integrifolia* globulin glycine-HCl eluted peak.

Isoelectric Focusing

When submitted to isoelectric focusing on 4% polyacrylamide 2.5% ampholine gel slabs (Fig. 9), the affinity chromatography peaks showed components with pI varying from around 4.6 to around 9.3. It was also noted that the several isolectins behaved differently.

DISCUSSION

The results of these experiments showed different behaviour of the albumins and globulins of the two seeds, when they were submitted to affinity chromatography on a human-stroma column.

The elution patterns obtained with the albumins from the two seeds are comparable but although the hemagglutinating active fractions are eluted in the same way (by decreasing the ionic strength) the lectin content in the two seeds are essentially different. The similarity of elution though suggests that they interact in the same way. However, the presence of only one active peak does not necessarily imply the presence of a single protein, since an isolectin family was already isolated from the albumin of *A. integrifolia* (MOREIRA and AINOUZ 1981).

The elution patterns of the globulins also showed a qualitative similarity. Both seeds presented the fractions eluted with H_2O (GII) with two distinct peaks. The two peaks are probably due to the presence of isolectins interacting differently with the stroma. In the fractions eluted with low pH, on the other hand, only one peak was observed in both seeds. The GIII peak of *A. integrifolia* contained more protein than the same peak in *A. incisa*, but the hemagglutinating activity was much lower in the *A. integrifolia* GIII. This low activity can be explained by the presence of an inhibitor, interacting with one of the terminals of the lectin, and detectable by SDS-poly acrylamide gel electrophoresis.

The two isolectin families presented many similarities. All the lectins presented the same subunits by SDS-polyacrylamide gel electrophoresis with or without beta-mercaptoethanol in the protein buffer, the only exception being fraction GIII from *A. integrifolia* that also contains a smear, probably due to an inhibitor. All the lectins were recognized by both rabbit antisera prepared against crude extract of each of the two seeds.

When the lectins of the two seeds are electrophoresed in an agarose gel containing either one or the other antiserum a fused rocket is obtained. But the several peaks presented a different behaviour by isoelectric focusing in polyacrylamide-ampholines gel slab.

From the results presented we suggest the existence of an isolectin family in each species, presenting the same antigenic sites and subunits but with slight differences in charge distribution.

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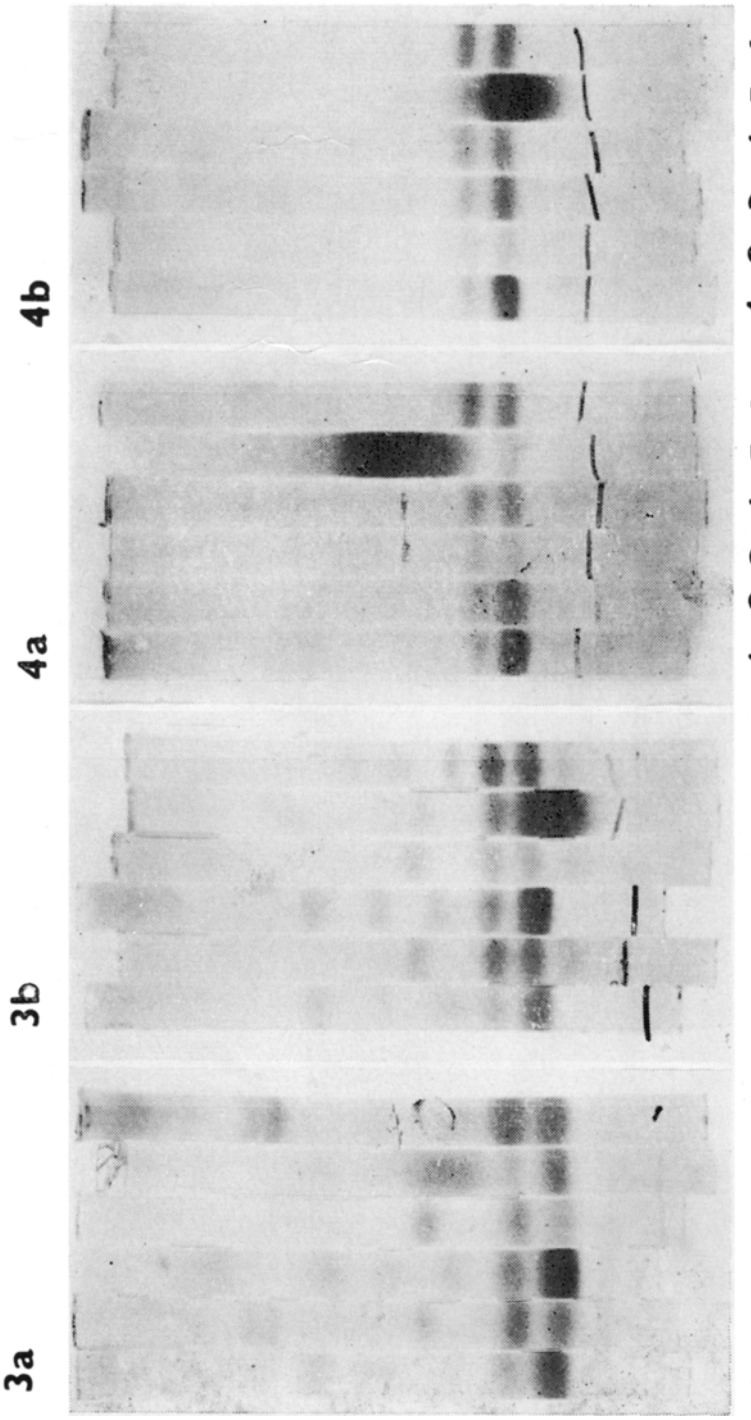
Figs. 3—9 at the end of the issue.



Figs. 3 and 4. Electrophoresis on SDS-polyacrylamide gel of untreated (a) and treated (b) *A. integrifolia* and *A. incisa* fractions respectively. — Fig. 3 Crude extracts (1, 2), albumins (3, 4) and globulins (5, 6). — Fig. 4. Peak AII (1, 2), peak GII (3, 4) and peak GIII (5, 6).

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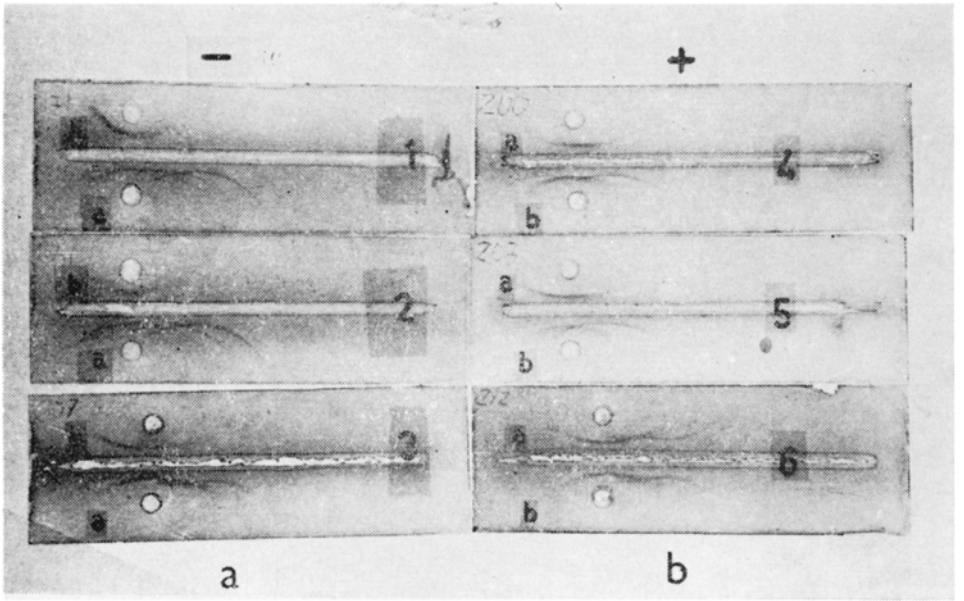

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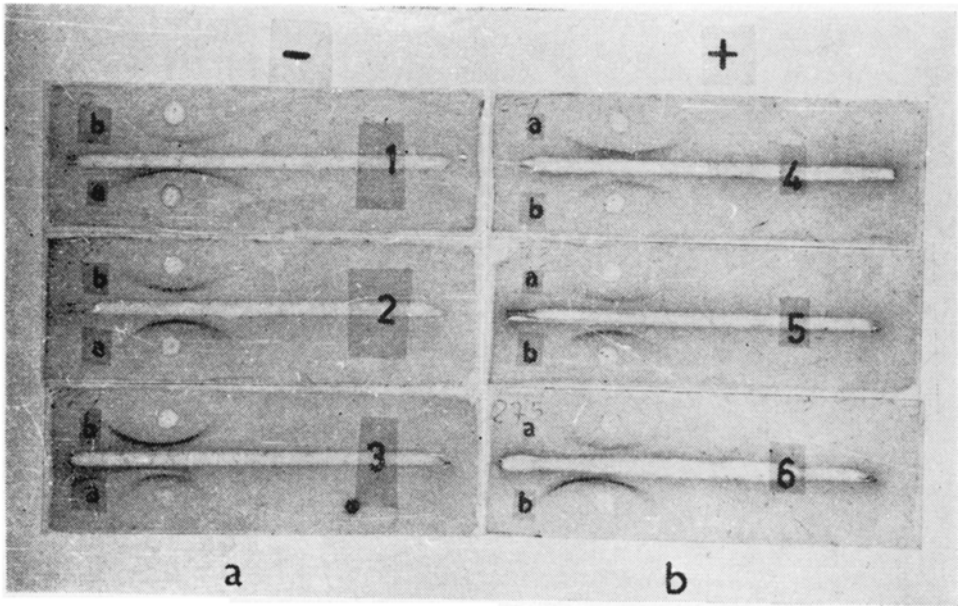

Figs. 5 and 6. Agarose gel immunoelectrophoresis of *A. integrifolia* (a) and *A. incisa* (b) fractions. The central troughs were loaded with *A. integrifolia* (1, 2, 3) and *A. incisa* (4, 5, 6) crude extract rabbit antisera. — Fig. 5. Crude extracts (1, 4), albumins (2, 5) and globulins (3, 6).
Fig. 6. Peak AII (1, 4), peak GII (2, 5) and peak GIII (3, 6).

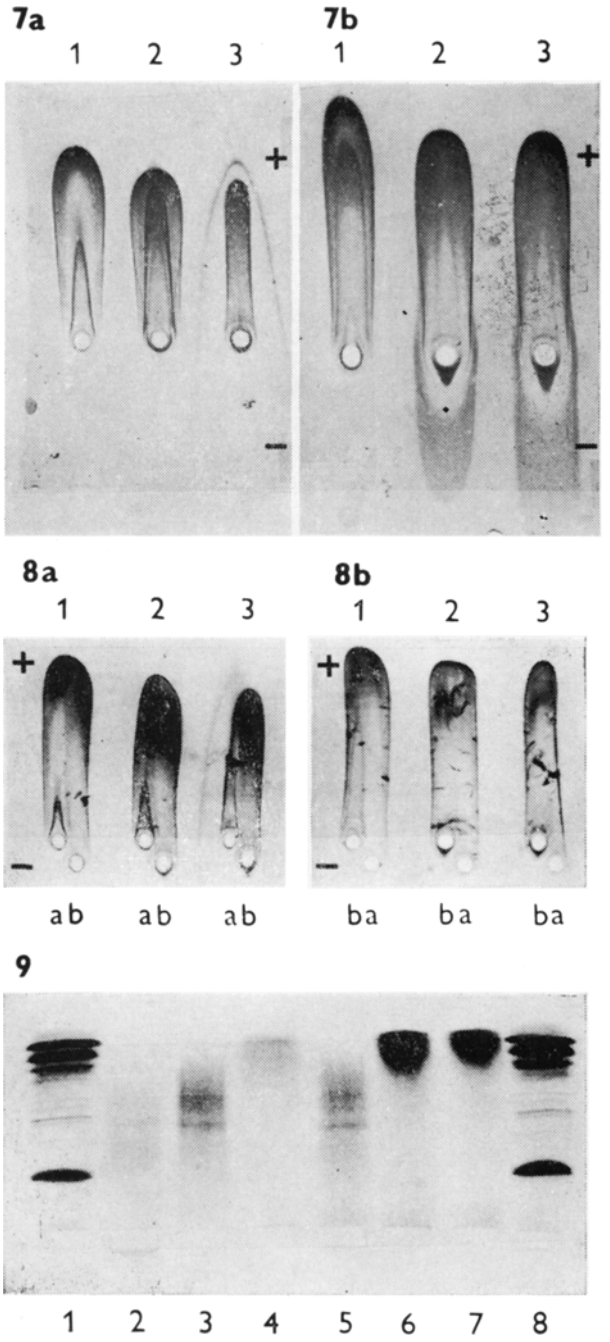

Fig. 7. Rocket immunoelectrophoresis on agarose gel of the *A. integrifolia* (a) and *A. incisa* (b), affinity chromatography peaks AII (1), GII (2) and GIII (3) respectively. The agarose plates contained *A. integrifolia* (a) and *A. incisa* (b) crude extract rabbit antisera.
Fig. 8. Fused rocket immunoelectrophoresis on agarose gel of *A. integrifolia* (a) and *A. incisa* (b) affinity chromatography peaks AII (1), GII (2) and GIII (3) respectively. The agarose plates contained *A. integrifolia* (8a) and *A. incisa* (8b) crude extract rabbit antisera.
Fig. 9. Isoelectric focusing on polyacrylamide gel slab. Samples 1 and 8, calibration mixture; 2, AII; 3, GII; 4, GIII of *A. integrifolia* and 5, AII; 6, GII; 7, GIII of *A. incisa*.