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ISOLECTINS FROM SEEDS OF *ARTOCARPUS LAKOOCHA*

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Key Word Index—*Artocarpus lakoocha*; Moraceae; lectin; isolectin; mucin.

Abstract—Two isolectins (ALA-I and ALA-II), were isolated from seed extracts of *Artocarpus lakoocha* by anion exchange chromatography on Q-Sepharose fast flow columns at pH 8.5 and 8.0. ALA-I was unbound to the column at pH 8.5 and moved towards the cathode in non-denaturing polyacrylamide gel electrophoresis, whereas ALA-II possessed opposite properties. The two *A. lakoocha* agglutinins appeared to be composed of two dissimilar subunits (α and β of M_r 14000 and 17200) bound non-covalently. The isolectins possessed several similar properties including: blood type agglutination; pH optimum; pH and temp stability; as well as binding specificity towards asialomucins.

INTRODUCTION

Lectins are a group of (glyco)proteins found in a wide variety of plants, animals and microorganisms that interact with glycoconjugates and polysaccharides by binding to specific carbohydrate residues. The presence of isolectins has been reported in various plants [1–4]. As part of a screening program for the occurrence of lectins in seeds of tropical plants in Thailand [5], we have found a lectin of *Artocarpus lakoocha*, which exhibits high haemagglutination activity. The present work demonstrates the presence of isolectins in the seeds of *Artocarpus lakoocha*. Purification and characterization of the isolectins with respect to some physico-chemical properties are also described.

RESULTS AND DISCUSSION

Artocarpus lakoocha agglutinin (ALA) is not organ specific. The haemagglutination activity of ALA was demonstrated in various organs of the plant except flesh. The highest and lowest activity was found in the seeds (14400 units per g fr. wt) and leaves (5 units per g fr. wt), respectively.

The isolectins of ALA were purified by ammonium sulphate precipitation and anion-exchange column chromatography (Q-Sepharose Fast Flow). Three protein peaks: I, II, and III which possessed haemagglutination activities were separated by the column at pH 8.5 (Fig. 1). Lectin of peak I proved to be the unbound fraction rather than the overloaded protein because the activity was still found in the washed fractions when this peak was reloaded to the column in the same condition. Lectins of peaks II and III were eluted from the column with 100 and 300 mM NaCl in 50 mM Tris–HCl, pH 8.5, respectively.

By non-denaturing polyacrylamide gel electrophoresis (ND–PAGE), two (N1 and N2) and four (N3–N6) protein bands were demonstrated in peaks II and III fractions, respectively (Fig. 2, lanes 3 and 4), whereas no protein band was observed in peak I fraction (Fig. 2, lane 2). However, lectin of peak I (designed ALA-I) was demonstrated by ND–PAGE running from anode to cathode (Fig. 2, lane 6).

Lectins of peak II were further purified by Q-Sepharose column, equilibrated with 50 mM Tris–HCl, pH 8.0. One major protein peak and one minor protein peak each with haemagglutination activities were found in the unbound and bound fractions, respectively (data not shown). The unbounded lectin designated ALA-II, corresponded to band N1 in the ND–PAGE (Fig. 2, lane 5).

We did not detect the small subunit peak of 20–21 amino acids as demonstrated for jacalin and *Maclura pomifera* agglutinin (MPA) [6]. However, smear diffused bands appeared close to the anode in the SDS–PAGE of the crude extract and the sample of peak III. They are possibly the small polypeptides found in other Moraceae lectins. These smaller chains perhaps play an important role in the heterogeneity of ALA, as postulated for jacalin and MPA [6]. Since there was only a very small amount of lectin activity (< 1%) remaining in this peak, therefore, no further attempts were undertaken to separate the lectins of this peak.

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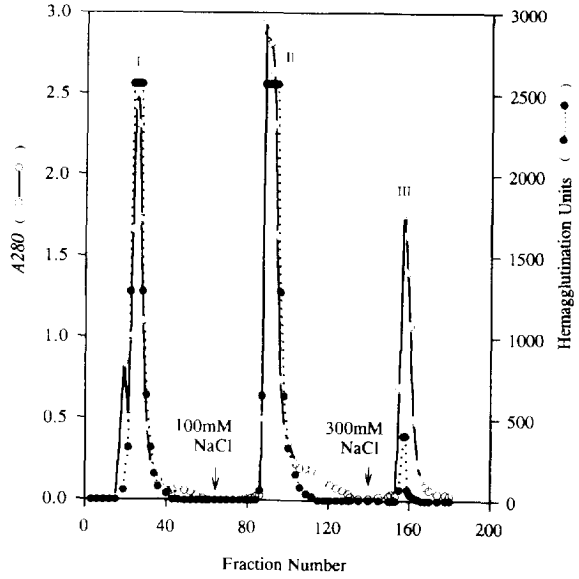

Fig. 1. Ion exchange chromatography of seed extract (50–80% satd $(NH_4)_2SO_4$) of *A. lakoocha*. Q-Sepharose fast-flow column was equilibrated with 50 mM Tris–HCl, pH 8.5. (○) *A* at 280 nm, (●) haemagglutination activity.

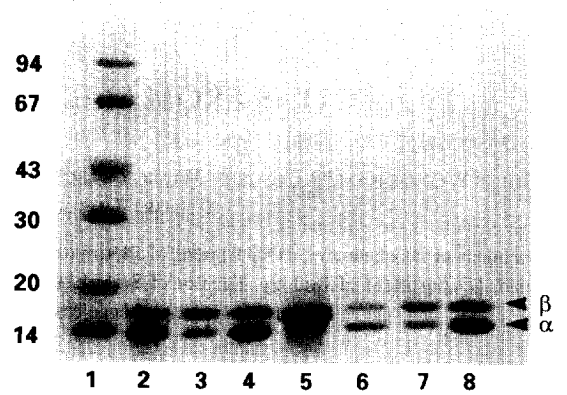

Fig. 3. SDS–PAGE of lectins from *A. lakoocha* seeds. (1) Standard M_r markers; (2) 50–80% satd $(NH_4)_2SO_4$; (3–5) peaks I (ALA-I), II and III of Q-Sepharose column pH 8.5, respectively; (6) ALA-II of Q-Sepharose column, pH 8.0; (7–8) ALA-I and ALA-II in the absence of 2-mercaptoethanol. The arrows indicate α- and β-subunits. The M_r marker of protein standards: phosphorylase B (94 k), bovine serum albumin (67 k), ovalbumin (43 k), carbonic anhydrase (30 k), Trypsin inhibitor (20 k) and α-lactalbumin (14 k).

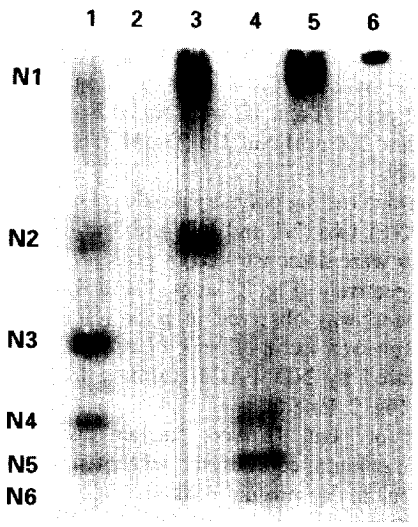

Fig. 2. ND–PAGE of lectins from *A. lakoocha* seeds at various stages of purification. (1) 50–80% satd $(NH_4)_2SO_4$; (2–4) peaks I (ALA-I), II and III of Q-Sepharose column pH 8.5, respectively; (5) ALA-II of Q-Sepharose column, pH 8.0; (6) ND–PAGE of ALA-I, running from anode to cathode.

In this study, at least two isolectins–ALA-I and ALA-II–were isolated. ALA-I differed from ALA-II in two categories: (1) ALA-I was unbound while ALA-II was bound to the Q-Sepharose column at pH 8.5; (2) ALA-I moved towards the cathode upon non-denaturing PAGE at pH 8.0), whereas ALA-II migrated towards the anode. Five isolectins have been reported for *Maclura pomifera* agglutinin, a member of related lectin of Moraceae family [7]. ALAs are not glycoproteins, since no glycoconjugate was detected using the phenol–sulphuric method [8]. The presence of isolectins in seeds of *A. lakoocha* might not arise from heterogeneity in the oligosaccharide chains as believed for various glycolectins [9]. The presence of two closely related ALAs presumably arises from variations in post-synthetic modifications, including cleavages, deamination of asparagine or glutamine side chain [10], or post-translational processing of a precursor species as speculated for jacalin and MPA [6].

Upon chromatography on the Q-Sepharose columns, it yielded 14 and 24 mg of ALA-I and ALA-II per g of seeds, which accounted for 18% and 32% (w/w) of total protein, respectively. ALA isolectins were composed of two noncovalently bound subunits: α and β of M_r 14 000 and 17 200, respectively (Fig. 3 lanes 3, 6 and 7, 8). However, the present study did not establish an accurate native or stoichiometry for the α and β subunits of ALA isolectins.

ALA-I and ALA-II possessed several similar properties. They both agglutinated red blood cells of several sources, including: human (groups A, B and O), rat, mouse, hamster, goose and pigeon, but not those of chicken. The isolectins were stable at pH 2.6–11.4, had optimum pH at 7.8–10.6 and did not require a divalent ion for their haemagglutination activities. Both lectins were heat labile, *ca* 5% and 20% activities of ALA-I and ALA-II remained after heating at 56% for 30 min, and were completely inactivated after heating at 80° for 60 min. In contrast to other *Artocarpus* lectins which possess specificity toward Gal or Galβ1-3GalNAc, both ALA isolectins did not respond to any of the sugars/derivatives tested. However, the isolectins were inhibited by *O*-glycosidically linked glycans (mucin, fetuin) which contain Gal-GalNAc in their molecular structures [11, 12], and were not inhibited by transferrin

Table 1. Carbohydrate inhibition of haemagglutination by *A. lakoocha* agglutinins

Glycoprotein	Minimal inhibitory concentration ($\mu g\,ml^{-1}$)
Asialo bovine submaxillary mucin	1.2
Bovine submaxillary mucin	1.8
Asialofetuin	12.5
Porcine stomach mucin	15.0
Fetuin	31.2

which is an N-linked glycan. Asialo forms of the glycoproteins tested showed greater inhibitory activities than their sialo forms (Table 1). Lectins which exhibit specificity toward glycoproteins and not sugars/derivatives have also been reported in the back of elderberry, *Sambucus sieboldiana* [1], and the tuber of *Alocasia indica* [4]. The structurally related isolectins of similar haemagglutinating and carbohydrate-binding properties has also been reported for lectins of wheat germ [2], *Phaseolus vulgaris* [3], MPA [7] and *Bandeir simplicifolia* [13].

Several properties of ALA isolectins reported in this study are different from Artocarpin characterized by Chowdhury *et al.* [14] as well as other lectins from the same genus. These discrepancies are possibly due to the genetic polymorphism or post-synthetic modification of the plant studied as mentioned above.

EXPERIMENTAL

Artocarpus lakoocha were collected locally in Khon Kaen University, Thailand.

Isolation and purification of the lectin. All the procedures described were carried out at 4–10° unless otherwise stated. Finely ground seeds (20 g) were homogenized in 200 ml of 50 mM Tris–HCl, pH 8.0 and stirred overnight. The homogenate was squeezed through cheesecloth and centrifuged at 12 100 g, for 20 min. The supernatant fluid was pptd between 50 and 80% satd $(NH_4)_2SO_4$. The ppt was collected, resuspended in 50 mM Tris–HCl, pH 8.5 and dialysed against several changes of the same buffer, overnight.

The first anion exchange chromatography was performed on the Q-Sepharose fast flow column, equilibrated with 50 mM Tris–HCl, pH 8.5. Dialysate of *ca* 200 mg protein was loaded at a flow rate of 20 ml hr^{-1}. The column was washed with the same buffer until the *A* at 280 nm of the effluent was negligible. The lectin which bound to the column was eluted step-wise with 180 ml each of equilibrating buffer, containing 100 and 300 mM NaCl, respectively.

The second anion exchange chromatography was performed on the same column and condition mentioned above, except the pH of the equilibrating buffer was 8.0.

Haemagglutination and carbohydrate inhibition. Haemagglutination activity was assayed using human blood group O erythrocytes unless otherwise stated. The erythrocytes were washed 3 times with 0.85% NaCl (NSS) and prepared as 2% (v/v) suspension. Two-fold serial dilution of the sample (50 μl)) with NSS was made in microtitre plates. Then, 50 μl of 2% erythrocytes suspension was added to each well. The activity was expressed as the reciprocal of the highest dilution showing positive agglutination after incubation at room temp. for 1 hr. The cationic dependence of the agglutination was tested in the presence of either 5 mM of $CaCl_2$, $MgCl_2$ or $MnCl_2$.

The inhibitory activity of carbohydrate was carried out as follows: to 25 μl of 2-fold serial dilutions of either sugar soln (400 mM) or glycoproteins (100 mg%) was added an equal vol. of 4 haemagglutinating doses of lectin. After 30 min of incubation, 50 μl of 2% erythrocyte suspension was added and the haemagglutination activity was scored after 1 hr of incubation. The maximum dilution of test soln showing haemagglutination-inhibition was recorded. The inhibitory activity was the lowest conc of carbohydrate soln that inhibited haemagglutination completely.

Polyacrylamide gel electrophoresis. SDS–PAGE in 7–18% polyacrylamide was carried out according to Ref. [15]. Proteins in the gel were stained with Coomassie brilliant blue R-250.

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