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α -D-Galactose-specific lectin from jack fruit *(A rtocarpus integra)* seed

G. SURESH KUMAR, P. S. APPUKUTTAN and DEBKUMAR BASU
Neurochemistry Division, Sree ChitraTirunal Institute for Medical Sciences and Technology, Trivandrum 695 011

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Abstract. An α-D-galactose-specific lectin from the seeds of jack fruit (*Artocarpus integra*) has been isolated in pure form by affinity chromatography on immobilised guar gum (a galactomannan). The lectin is shown to be a glycoprotein containing 3% carbohydrate and having a molecular weight of 39,500 as determined by gel filtration. Sodium dodecyl sulphate gel electrophoresis revealed a single polypeptide of 10,500 dalton, indicating that the native lectin is a tetrarner of identical subunits. The hemagglutinating activity of the lectin towards erythrocytes of all blood groups is found to be the same.

Keywords. Jack fruit seed; lectin; α-D-galactose; guar gum; affinity chromatography.

Introduction

Lectins are proteins of non-immune origin, present in plant extracts and they exhibit cell agglutinating property. They are widely distributed in nature, being found in plants, micro-organisms and animals. They bind mono- and oligosaccharides with remarkable specificity in the same way as the enzmes bind substrates and antibodies bind antigens. Although the physiological function of plant and animal lectins is unknown, these ubiquitous carbohydrate-binding (glyco) proteins can recognise and bind to complex carbohydrates as they occur in solution or on membranes and cell surfaces (Lis and Sharon, 1973; Goldstein and Hayes, 1978). There is increasing indication that they function in both intercellular and intracellular recognition phenomena in microorganisms, plants and animals (Boyd, 1970; Goldstein *et al.,*1968; Simpson *et al.,* 1978). This laboratory has been investigating this important class of proteins present in the locally available seeds, and the purification of N-acetyl-D-galactosamine-specific lectin from winged bean has been reported (Appukuttan and Basu, 1981). In this paper we describe the purification and physicochemical properties of an α-galactose-specific lectin from jack fruit seed *(Artocarpus integra)*. Jack fruits are typical tropical seasonal fruits. Their seeds contain very little fat but contain about 40% carbohydrates and 7% protein.

Abbreviations used: Con A, Concanavalin A; PBS, 20 mM potassium phosphate buffer pH 7.4 containing 150 mM NaCl.

Materials and methods

Jack fruit seeds were collected from locally available sources. Crystalline bovine serum albumin, guar gum, Tris, acrylamide, N,N,N′ N′ -tetramethylene diamine, N,N-methylene bis acrylamide, sodium dodecyl sulphate, 2-mercaptoethanol, Coomassie Brilliant Blue and α-methyl glucoside were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. The molecular weight standards were purchased from Pierce Chemical Co., Rockwell, Illinois, USA. Biogel P-100 of Bio-Rad was a gift from Dr Krishna Bakshi of the National Institutes of Health, Bethesda, Maryland, USA. Sepharose 4B and Blue Dextran 2000 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden, *p*-Nitrophenyl derivatives of α-D-galactose and □-D-galactose were purchased from Koch-Light Laboratories, Colnbrook, England. All other chemicals used were Analytical reagent grade. Blood samples were obtained from our Institute's Blood Bank.

Guar gum was insolubilized by cross-linking with epichlorohydrine in alkaline medium according to Appukuttan *et al.* (1977). The protein was estimated with crystalline bovine serum albumin as standard according to the method of Lowry *et al.* (1951). Concanavalin A (ConA) was isolated by the method of Surolia *et al.* (1973). The Con A was immobilized on CNBr-activated Sepharose 4B by the method of Bishayee and Bachhawat (1974).

Polyacrylamide gel electrophoresis was done according to Davis (1964). SDS-polyacrylamide gel electrophoresis was carried out at pH 7.0 (Weber and Osborn, 1969). The molecular weight of native protein was estimated by gel filtration on Biogel P-100 according to Andrews (1965). Neutral sugar was estimated by the phenokulphuric acid method of Dubois *et al.* (1956).

Purification of jack fruit seed agglutinin

All operations were carried out at 0–4°C unless otherwise mentioned. Dehusked seeds (10 g) were soaked for 24 h in 20 mM potassium phosphate buffer, pH 7.4 containing 150 mM NaCl (PBS). The seeds were cut into small pieces and homogenised with 70 ml of PBS in a Sorvall omnimixer for 3 min at maximum speed. The homogenate was stirred for 3 h and centrifuged at 20,000 *g* for 15 min. The precipitate was discarded and the supernatant fluid made to 70% saturation (49 g/100 ml) with solid ammonium sulphate. The suspension was stirred for 60 min and the precipitate was collected by centrifugation as before. The ammonium sulphate precipitate was dissolved in 10 ml of PBS and dialysed with 2 changes against the same buffer for 16 h. Any precipitate formed during dialysis was discarded by centrifugation as before.

The dialysed supernatant was absorbed on a cross-linked guar gum column (2×15 cm). The column was equilibrated with PBS and washed with the same buffer till the absorbance at 280 nm of the effluent was lower than 0.05. The agglutinin was then eluted with PBS containing 150 mM galactose and 10 ml fractions were collected. The active fractions (No. 30 to 36) were pooled and dialysed against PBS with several changes for 24 h.

Results and discussion

The cross-linked guar gum retained a portion of the ammonium sulphate-precipitate, which was eluted only with galactose (figure 1). The binding capacity of

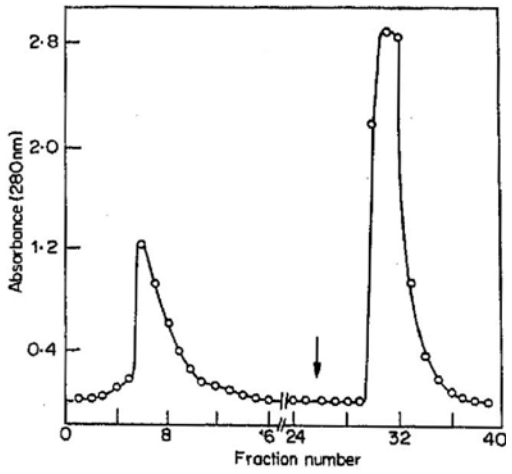

Figure 1. Affinity chromatography of jack fruit seed lectin on cross-linked guar gum. Details are given under materials and methods.

the matrix was found to be 5 mg lectin protein per ml of packed column. More than 50% of the applied protein was found to constitute the lectin. In a separate experiment, the lectin content was found to be 1.2% (by weight) of the seed. The galactose-specific eluate of the protein in disc gel electrophoresis showed a single band at pH 4.5 (figure 2). The protein failed to move at alkaline pH at 5%, 7.5% and 10% acrylamide concentrations.

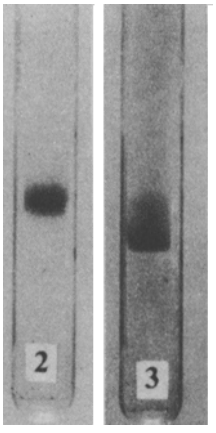

Figure 2. Polyacrylamide gel electrophoresis of purified jack fruit seed lectin at pH 4.5. 30 µg protein was used in 7.5% acrylamide gel.

Figure 3. SDS-polyacrylamide gel electrophoresis of jack fruit seed lectin. 30 µg protein was used in 10% acrylamide gel.

The remarkable capacity of guar gum, a galactomannan to bind α-galactose-specific lectin was demonstrated by the isolation of *Ricinus communis* lectins (Appukuttan *et al.,* 1977). Guar gum contained β-(1→ 4) linked mannose units in the main chain with a single galactose moiety linked α-(1→ 6) to every alternate mannose unit (Dea and Morrison, 1975). Due to this structure it has 100-fold increased capacity to bind *Ricinus communis* lectin compared to Sepharose (Appukuttan *et al.,* 1977). The reason for the high binding capacity with the present lectin may be the same. A comparison between the binding affinities of α- and β-linked galactose moieties of *p*-nitrophenylderivatives for lectin could not be made from erythrocyte agglutination studies due to the poor solubility of *p*-nitrophenyl galactoside in PBS. However the α-isomer was found to be 20 times more inhibitory when soluble guar gum was used in place of erythrocytes (data not shown). This difference also explained the high affinity of the lectin towards guar gum and its inertness towards Sepharose. This marked preference for the α-anomer over the β-anomer is not observed in other predominantly α-galactoside binding lectins. Thus in the case of *Bandeirae simplicifolia* I lectin (Hayes and Goldstein, 1974) and pea nut (*Arachis hypogaea*) lectin (Lotan *et al.,*1975), the α-galactoside moieties are only 1.3 and 1.5 times better inhibitors than the β-anomers, respectively.

The lectin did not show specificity towards any blood group in hemagglutination reactions. The erythrocytes from all the blood groups were agglutinated with equal efficiency. In order to compare the avidity of various sugars towards the lectin, their capacities to inhibit hemagglutination on prior incubation with lectin was studied. Making two-fold serial dilutions, the minimum concentration of each sugar that inhibits twice the minimum hemagglutinating amount of lectin was determined (table 1). N-acetyl D-galactosamine, though 4 times better compared

Table 1. Inhibition capacity of common sugars on agglutination of erythrocytes by jack fruit seed agglutinin.

Sugar	Minimum concentration (mM) required to inhibit twice the hemagglutinating amount of lectin.
Glucose	N.I.[a]
Galactose	200
Mannose	N.I.[a]
Methyl-α-D-glucoside	N.I.[a]
Methyl-α-D-galactoside	6
Methyl-α-D-Mannoside	N.I.[a]
p-Nitrophenyl-α-D-galactoside	0.75
p-Nitrophenyl-β-D-galactoside	N.I.[b]
D-Galactosamine	400
N-Acetyl D-galactosamine	50

Aliquots of 0.2 ml PBS containing 150 μg lectin (double the minimum amount for hemaglutination) with or without serial dilution of the sugars were incubated for an hour at 5°C and 0.05 ml of a 5% suspension of erythrocytes in PBS was added. The mixture was kept at 25°C with occasional shaking and agglutination noted after 2 h.

[a] No inhibition upto 400 mM concentration. [b] No inhibition at the limit of solubility.

to galactose, was a very poor inhibitor, compared to *p*-nitroprienyI-α-D-galactoside or methyl-α-D-galactoside.

The molecular weight determination of native lectin by gel filtration on Biogel P-100 gave a value of 39,500 daltons. However, SDS-polyacrylamide gel electrophoresis with or without 2-mercaptoethanol showed a single band corresponding to a molecular weight of 10,500 (figure 3). It is reasonable to conclude that the lectin is a tetramer, in which 10,500 dalton polypeptides are non-covalently linked. This structural phenomenon is similar to that of concanavalin A. The present lectin showed no metal requirement for its sugar binding activity.

The homogeneous lectin after guar gum affinity column elution was passed through a concanavalin A Sepharose column and the column was thoroughly washed with PBS and then eluted with the same buffer containing 500 mM α-methyl glucoside. The hemagglutinating activity was associated with the protein fraction eluted with α-methyl glucoside, indicating that the lectin is a glycoprotein. Estimation by the phenol-sulphuric acid method with glucose as standard showed 3% neutral sugar content in the lectin.

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