A new unusual galactose-specific lectin from the seeds of Indian lablab beans

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Seeds of the Indian lablab beans (*Dolichos lablab* var. *typicus*) contain a glucose/mannose-specific lectin that was affinity-purified and well characterized from our laboratory[1]. The seeds were also found to contain a protein that strongly agglutinates only rabbit erythrocytes. The agglutinating activity is inhibited only by galactose and its derivatives. However, this newly found lectin fails to bind on the sepharose–galactose columns, and hence was isolated by gel filtration on Biogel P-200. The lectin eluted from this column with a molecular size of 1,20,000 and was found to be homogeneous in native PAGE. In SDS-PAGE the lectin dissociated into two subunits with apparent molecular masses of 48 and 20 kDa, respectively. Alanine was the only detectable amino terminal amino acid. The lectin was found to be a glycoprotein with 3% neutral sugar and cross-reacts with an antibody to the well-characterized glucose/mannose specific seed lectin.

INTEREST in the area of plant lectins is largely due to the diverse applications these proteins have in various fields of modern biology. Although several lectins have been purified and well characterized, their precise functions still remain unclear[2]. Our laboratory has been interested in affinity purification and characterization of new lectins from plant and animal species with a long-term objective to understand their structure–function relationships[3,4]. While working with the seed extracts of the lablab beans, we detected that they contain in addition to the well characterized glucose/mannose-specific lectin, another lectin whose agglutinating activity is inhibited by galactose. From the seed extracts both the glucose/mannose-specific and the galactose-specific lectins can be completely separated by ammonium sulphate precipitation. The 30–60% fraction contains largely glucose/mannose-specific lectin[1] while the 60–80% fraction contains only galactose-specific lectin.

In order to isolate the galactose-specific lectin, we prepared sepharose–divinyl-sulfone-galactose and sepharose–divinyl-sulfone-lactose gels which can efficiently be used for the purification of the galactose-specific lectin from *Momordica charantia* seeds[5]. The 60–80% ammonium sulphate fraction obtained from the lablab bean seed extract was first passed through sepharose–mannose gel (all the protein loaded was recovered in the unbound fraction, suggesting that this fraction is devoid of any glucose/mannose-specific lectin). The protein recovered was then applied to sepharose–galactose and sepharose–lactose gels and the lectin activity could not be bound onto these gels and was recovered completely in the unbound fractions. Similar results were obtained when cross-linked guargum gel used for the purification of galactose-specific lectins[6] was employed. In order to isolate the lectin in pure form, we passed the 60–80% fraction on Biogel P-200 equilibrated with 25 mM Tris-HCl buffer pH 7.4, containing 150 mM sodium chloride. The protein eluted into two peaks. Only peak II showed lectin activity (Figure 1) which was pooled and used in all studies. From 7 g of the seed meal, 50 mg of protein could be recovered in the 60–80% ammonium sulphate fraction. From this the lectin recovered was about 7 mg. The native molecular size of this protein was found to be 1,20,000. Protein from peak II when analysed by PAGE (ref. 7) showed a single band (Figure 2 *a*) and in SDS-PAGE (ref. 8) it dissociated into two subunits under reducing conditions with apparent molecular masses of 48 and 20 kDa respectively (Figure 2 *b*). Many other galactose binding lectins from different seeds such as the soybean, *Crotolaria juncea*[9] that have been affinity-purified, exhibit a native molecular mass of 1,20,000 and are tetrameric in nature with subunit molecular size of 30,000 and are rich in acidic amino acids. However the lectin from *Momordica charantia*, although tetrameric in nature and affinity-purified, is made of two types of subunits, 28 and 30 kDa respectively[10]. On the other hand, the lectins from different erythrina seeds exhibit a native molecular mass in the range of 56,000 to 68,000 and are composed of two noncovalently-linked subunits[11]. These lectins were all found to be also glycoproteins whose activity is inhibited by galactose and its derivatives.

The lectin isolated in this study from the lablab beans was found to be a glycoprotein with 3% carbohydrate as analysed by phenol sulphuric acid method[12]. Amino terminal analysis of the purified lectin using DABITC reagent[13] revealed alanine as the only amino terminal amino acid. The lectin did not agglutinate untreated and trypsin-treated human ABO erythrocytes, but agglutinated only untreated and trypsin-treated rabbit erythrocytes. Ten µg of protein was sufficient to cause agglutination of trypsin-treated erythrocytes. As the lectin failed to bind on the conventional affinity matrices tested and was found to agglutinate only rabbit erythrocytes, we prepared membrane proteins from rabbit erythrocytes following the protocol described for preparation of human A erythrocyte membrane glycoproteins[14]. The membrane proteins obtained were

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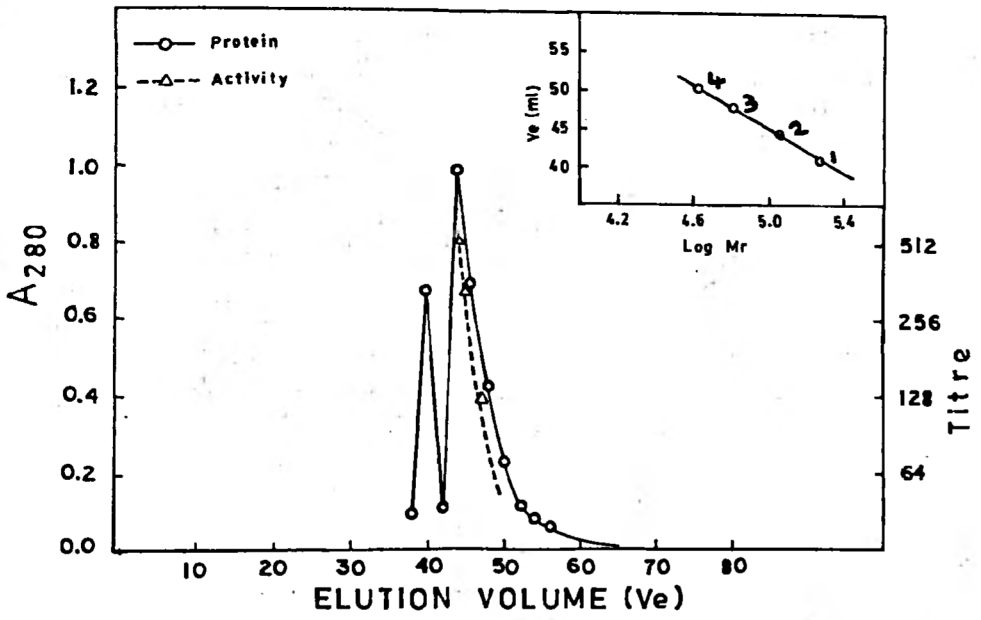

Figure 1. Gel filtration of the lablab bean galactose-specific lectin. The 60–80% ammonium sulphate fraction protein (20 mg) was dissolved in 1.5 ml of 25 mM Tris-HCl buffer pH 7.4 containing 0.15 M sodium chloride and applied to a Biogel P-200 column (1.8 × 86 cm) equilibrated with the same buffer. Fractions 2.0 ml and absorbance measured at 280 nm. Inset. Plot of elution volume (Ve) vs log Mr.(o) standard. 1, myosin (205,000); 2, β-galactosidase (116,000); 3. Bovine serum albumin (66,000) and 4, Ovalbumin (45,000).

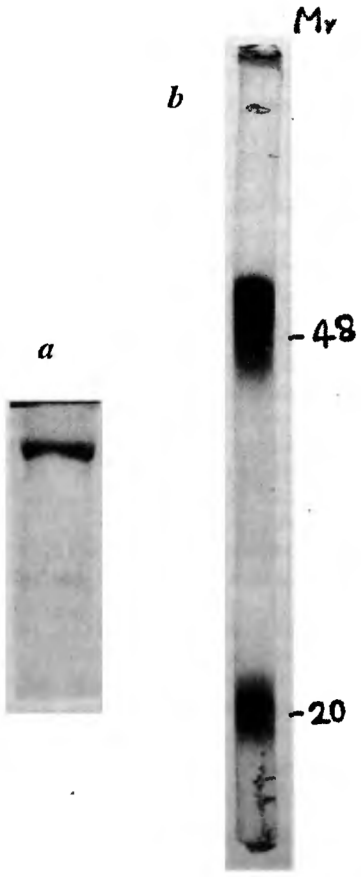

Figure 2. *a*, Native PAGE of the purified lectin. 7.5% gel Tris-HCl pH 8.9. *b*, SDS-PAGE of the purified lectin under reducing conditions.

concentrated and coupled to Affigel-15 (containing a 15 carbon spacer between the gel and the matrix) following manufacturer's instructions. When the 60–80% ammonium sulphate fraction containing the galactose-binding lectin was passed through this gel matrix, no protein could be retained on this gel also suggesting that this lectin is different from other galactose-specific lectins described. Among a number of sugars tested for the inhibition of lectin activity, only galactose, its derivatives and lactose were found to be inhibitory (Table 1). Neither glucose nor mannose was inhibitory to the lectin activity. It is apparent from the table that presence of a hydrophobic group in the first or the sixth position in galactose, enhances the affinity of the sugar to the lectin. Many galactose-specific lectins purified to date show similar inhibitory effects[15,16]. The general properties of the purified lablab bean galactose lectin such as the sugar specificity, native molecular size, tetrameric and glycoprotein nature seem to be similar to the other well characterized galactose-specific seed lectins[9–11]. The only unusuality observed is its nonbinding to the conventionally-used affinity matrices such as sepharose–galactose, sepharose–lactose and guargum gels that have high potential to purify galactose-specific seed lectins.

When the immunoreactivity of this protein was analysed in an immunodiffusion experiment, using an antibody to the purified glucose/mannose-specific seed lectin, the lectin clearly cross-reacted with the antibody, suggesting similar antigenic sites among these two lectins (Figure 3). Very few legume species have been

Table 1. Inhibition by various saccharides, the haemagglutinating activity of the galactose-specific lectin from the seeds of Indian lablab beans using trypsin-treated rabbit erythrocytes. 0.01 to 0.1 ml of stock 0.1 M sugar solutions were used

Sugar	Inhibitory concentration (mM)
Galactose	5.0
Lactose	5.0
Methyl-D-galactopyranoside	2.5
6-O-Methyl galactopyranoside	2.5
N-Acetylgalactosamine	2.5
N-Acetylglucosamine	NI
Mannose	NI
Glucose	NI
Fucose	NI
Cellobiose	NI

NI, Not inhibitory.

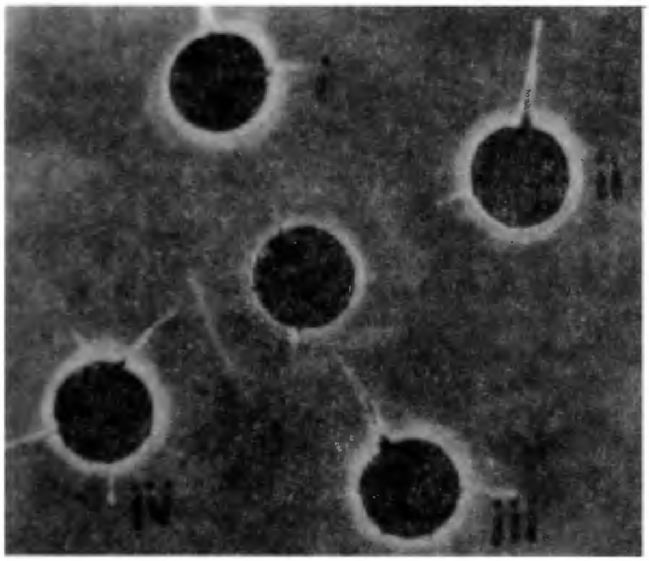

Figure 3. Immunodiffusion of the glucose/mannose-specific lectin and galactose-specific lectin from the seeds of lablab beans. Central well contained 20 µl of rabbit antiserum to the affinity-purified glucose/mannose-specific seed lectin. Wells i and iii contained purified glucose/mannose-specific seed lectin, and wells ii and iv contained purified galactose-specific seed lectin.

reported to contain more then one type of seed lectin. Seed lectins purifed from *Vicia cracca* and field bean were found to be distinct in their physico-chemical and biological properties[17,18]. Despite the differences in the sugar specificities of the two lectins from the lablab beans, it is interesting to note the antigenic similarity between them. When the seeds were grown into plants, the stems and leaves contained protein that strongly agglutinates also only rabbit erythrocytes whose activity is again inhibited by galactose and its derivatives and not by glucose/mannose. It also shows cross-reactivity with the glucose/mannose-specific seed lectin antibody (data not shown). This lectin, like the galactose-specific seed lectin, fails to bind to galactose and lactose matrices, as well as to the rabbit erythrocyte membrane glycoproteins coupled to Affigel matrix[19]. These data further suggest that only the galactose-specific lectin is expressed in the plant although the seeds contain both glucose/mannose specific and galactose-specific lectin.

In summary, this study clearly demonstrates the appearance of two distinct lectins in the seeds of the lablab beans that are immunologically related, although they differ in their native and subunit patterns. This new information gives enough scope to investigate in detail, the structural analysis of the galactose-specific seed lectin which can lead to understand the primary sequence relationships among the glucose/mannose-specific lectin and the galactose-specific seeds lectin in view of the immunological cross-reactivity exhibited.

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