

Studies on lectins from *Abrus precatorious* L

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ABSTRACT

Lectins are proteins of non-immune origin that recognise carbohydrates in a very specific way and can be used as probes to detect different types of glycoconjugate molecules in cells. The lectin is a metalloprotein that strongly agglutinates rabbit erythrocytes and is not inhibited by various simple sugars. Lectins are a heterogeneous class of proteins (or glycoproteins) that recognize carbohydrate moieties of glycoconjugates with high specificity and bind to them in a reversible way without altering their covalent structures. The purified lectin contains carbohydrate. A lectin from *Abrus precatorious* seeds was extracted by treating seed meal (50g) with Tris HCl and purified by affinity chromatography on Sephadex G-50 column. Subunit molecular mass was determined and fragments were estimated by SDS-PAGE. Haemagglutination assays were carried out in microtitration plates. Only one protein band with an apparent molecular mass of 32kDa was found.

Key words: *Abrus precatorious*, lectin ,Seed protein.

INTRODUCTION

The lectins represent a large group of plant proteins. Lectins have been found in less than 500 species, which indicates that only a limited number of higher plants contain detectable levels of lectins (Van Damme *et al.*, 1998b). The concentration of lectins in seeds varies considerably (from 1 to 10% of the total seed protein). In some species, values to 50% have been reported, whereas in others lectins are barely detectable with techniques currently used (Peumans and Van Damme, 1998). Seeds from plants of the family Leguminosae are particularly rich in lectins, their concentration being as high as 10% of the total nitrogen of mature seed extracts (Etzler, 1986). Lectins constitute a group of proteins or glycoproteins of non-immune origin, and they bind reversibly to carbohydrates and usually agglutinate cells or precipitate polysaccharides and glycoconjugates [Goldstein *et al.*, 1980]. The main characteristic of this class of proteins is their ability to interact with carbohydrates and thus combine with

glycocomponents of the cell surface, as well as with cytoplasmatic and nuclear structures and the extracellular matrix of cells and tissues from throughout the animal and plant kingdoms, down to bacteria and viruses (Leathem and Brooks, 1998). In fact, these proteins are very useful tools in immunology and cell surface research. Due to their chemical properties, lectins attract a great deal of attention in the fields of immunology, cell biology, membrane structure, cancer research and genetic engineering, etc. In recent years, there have been many reports on the antimicrobial and pesticidal activities of lectins, and the application of lectins in agriculture (Chrispeel and Raikhel, 1991; Peumans and Van Damme, 1995). The isolation and characterization of novel lectins reveal properties, which are of practical importance for different areas of biological research. Therefore, the objective of this work was to isolate and characterize a lectin from *A. precatorious* seeds. In this paper we report the purification and some properties of a lectin from the seeds of *A. recatorious* a legume that grows in Marathwada region of Maharashtra.

MATERIAL AND METHODS

Abrus precatorious L seeds were collected from plants growing in the garden. The botanical identification was carried out at the Department of Biotechnology, Government institute of science Aurangabad.

Seeds of *Abrus precatorious* were finely ground in a coffee mill and extracted in 0.15 M NaCl (1:10, m/v) at room temperature for 4 h. The extract was centrifuged (10,000 g, 20 min, 5°C) and the resultant supernatant applied to a Sephadex G-50 column (400 × 25 mm) equilibrated with 0.15 M NaCl containing 5 mM CaCl₂ and 5 mM MnCl₂. After removing unbound material, the lectin was eluted with the equilibration solution containing 0.1 M glucose. The lectin was dialysed, freeze-dried, and stored at 5°C.

The measurement of protein concentration in the different fractions was performed according to Bradford (1976), using bovine serum albumine (BSA) as standard. The absorbance at 280 nm was used to estimate the protein concentration in column eluates.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDSPAGE) was performed in 2 mm thick vertical slab gels, according to the method of Laemmli (1970), using 3.95% and 12.5% stacking and running gels, respectively. Samples were dissolved in 0.0625 M Tris-HCl pH 6.8, containing 2% SDS buffer and 1% β-mercaptoethanol then incubated at 100°C for 10 min. Electrophoresis was carried out at a constant current of 50 mA for 2h. The protein bands were visualized by staining the gel with Coomassie Brilliant Blue-R 250.

Effect of EDTA was done by measuring the lectin haemagglutinating activity after dialysis of the native protein against 0.2 M EDTA in 0.15 M NaCl for 48 h at 4°C. The recovery of activity was determined by posterior addition of different concentrations of CaCl₂ and MnCl₂ (0 to 10 mM). Immunochemical studies were performed to establish the relationships between *A. precatorious* and other lectins. Immunodiffusion tests were carried out on 1% agarose gels prepared with 0.05 M Tris-

barbital buffer pH 8.0 containing 0.02% sodium azide (Clausen, 1969). Neutral sugar content of the purified lectin was estimated by the phenol sulphuric acid method (Dubois *et al.*, 1956) with glucose as standard.

Lectin samples were hydrolysed (in sealed glass tubes under N₂ atmosphere) for 24, 48 and 72 h at 110°C in 6 M HCl. After hydrolysis, HCl was removed by evaporation and the residue was analysed in amino acid analyser.

RESULTS AND DISCUSSION

Gel electrophoresis in presence of SDS and 2-mercaptoethanol was used to estimate the molecular weight of the lectin subunits. Polyacrylamide gel electrophoresis of the lectin, treated with SDS and β-mercaptoethanol gave only one protein band with an apparent molecular mass of 32 kDa.

The seed flour was extracted with 0.15 M NaCl, centrifuged and the clear supernatant fractionated by treatment with ammonium sulphate to saturation levels. The seed flour contains 40% protein on a dry weight basis and a high lipid content.

The dialysis of the lectin against 0.2 M EDTA in 0.15 M NaCl caused a total loss of the haemagglutinating activity. The full haemagglutinating activity of the lectin was recovered by addition of Ca²⁺ and Mn²⁺ at different concentrations (1, 3, 5, 7, and 10 mM). In fact, The optimum concentration for maximum hemagglutinating activity was 3-5 mM for both cations. Similar results were observed for *Dioclea altissima* lectin (Moreira *et al.*, 1996). The requirement for metal is a general physico-chemical property of most legume lectins (Goldstein and Poretz, 1986; Sharon and Lis, 1990).

The purified lectin agglutinated rabbit erythrocytes and also agglutinated human erythrocytes. On the other hand, goat erythrocytes were not agglutinated at all. Generally *A. precatorious* are not specific for any type of red blood cells (Goldstein & Poretz, 1986). Sugar moiety was found on *A. precatorious* lectin by the phenol-sulphuric acid method suggesting that this lectin is

a glycoprotein. As lectin from *Dioclea lehmanni* seeds is not glycoprotein (Perez et al., 1990). The immunodiffusion studies showed that *Abrus precatorious* lectin exhibits a complete

immunological identity to other lectins assayed. Further structural and functional characterization will be necessary for a better understanding of *A. precatorious* lectin properties.

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