ABSTRACT

It has been postulated that lectins mediate rhizobium-legume symbiosis through interaction of extracellular polysaccharides with root hair cells. A number of leguminous lectins have been studied in detail to explore the exact physiological role of lectins in biological system. In view of this, we have isolated a lectin from Phaseolus mungo seeds in essentially pure form. The results include some of its important physico-chemical and carbohydrate binding properties whose delineation would form an essential prerequisite for the proper understanding of the role of lectin in the plant physiology.

In order to isolate the lectin, seed homogenate was prepared in 0.01 M Tris-HCl buffer, pH 7.4 containing 0.25 M NaCl (operating buffer). The homogenate was acidified with 0.3 M acetic acid to pH 4.0 for overnight at 7°C. The extraneous proteins were removed by centrifugation. The supernatant was dialysed against operating buffer and subjected to affinity chromatography on galactosyl Sepharose 6B column (4 x 8.8 cm) equilibrated with the operating buffer. The lectin was specifically eluted with 0.25 M galactose. The lectin showed hemagglutination of trypsinized rabbit erythrocytes which was specifically inhibited by galactose. The yield of
lectin was found to be about 0.8% with respect to the total protein present in acid homogenate.

The affinity purified lectin moved essentially as a single protein band in sodium dodecyl sulphate polyacrylamide gel electrophoresis. In addition, gel chromatography of the lectin on Sephadex G-150 column (1.78 x 99 cm) gave one major symmetrical peak, suggesting that the lectin preparation was homogeneous with respect to size. The relative mobility of lectin in presence and absence of 2-mercaptoethanol was found to be 0.39 which corresponded to a molecular weight of 66,000. The native molecular weight of lectin as determined by analytical gel chromatography on Sephadex G-150 column (1.78 x 99 cm) was found to be 137,000 which suggested that the *Phaseolus mungo* lectin consists of two identical subunits which are held together by non-covalent forces.

The ultraviolet absorption spectrum and fluorescence excitation and emission spectra were measured in 0.01 M Tris-HCl buffer, pH 7.4 containing 0.25 M NaCl. The lectin absorbed maximally near 278 nm. The excitation and emission maxima were found to occur near 278 nm and 334 nm respectively. The lectin was found to be a glycoprotein in nature with 8.3% neutral carbohydrate content. The protein was devoid of sialic acid residues. The values of Stokes (iv)
radius, diffusion coefficient and frictional ratio, determined by gel chromatography on Sephadex G-150 column were found to be 4.3 nm, $5.18 \times 10^{-7}$ cm$^2$ sec$^{-1}$ and 1.28 respectively. The deviation from globular shape may be attributed to glycoprotein nature of the lectin.

The *Phaseolus mungo* lectin readily caused agglutination of trypsinized rabbit erythrocytes which was found to be specific. Thus, hemagglutinating activity of lectin was measured against trypsinized rabbit erythrocytes by hemagglutination assay. The hemagglutinating activity of lectin was found to be sensitive to heat treatment. The saccharide binding specificity of lectin was investigated in presence and absence of ten different sugars. The inhibition of hemagglutination as a function of increasing concentration of saccharide was used to determine the concentration of saccharide required for 50% inhibition of the lectin activity i.e. $C_M$ value for each sugar was determined. The results showed that *Phaseolus mungo* lectin isolated in this study was galactose specific. The lectin also showed affinity for N-acetylgalactosamine. The $C_M$ value for N-acetylglactosamine (17 mM) was found to be more than galactose (9.5 mM), which indicated that the lectin was more specific for galactose than for N-acetylglactosamine. The lectin showed higher affinity for melibiose ($C_M = 6.1$ mM).
whereas glucose, sucrose and lactose had virtually no effect on lectin induced hemagglutination. Further, in the concentration range used in this study, galactosamine did not significantly affect hemagglutinating activity of the lectin. The derivatives of galactose i.e. methyl α-galactoside, methyl β-galactoside and p-nitrophenyl α-galactoside were also tested for their inhibitory activity. It was found that the methyl α-galactoside was the most potent inhibitor of hemagglutination ($C_M = 2.9\text{ mM}$) followed by p-nitrophenyl α-galactoside ($C_M = 4.2\text{ mM}$), whereas methyl β-galactoside was a non-inhibitor ($C_M > 10.7\text{ mM}$) in the concentration range used in this study (0-10.7 mM). This indicated that the lectin preferentially interacted with α-glycosides. Further, the observation that p-nitrophenyl α-galactoside was slightly less effective inhibitor than methyl α-galactoside indicated the absence of any hydrophobic binding site adjacent to the saccharide binding site on the lectin.

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