SYNOPSIS

The ubiquitous occurrence of lectins is a well-established fact. They occur in microorganisms, animals, plants, but are readily detectable in plants seeds and are widely used in carbohydrate biochemistry, cell biology, e.g. separation of various biologically active compound and cells. Plant lectins have been widely used for the detection, isolation and characterization of glycoconjugates using their characteristic carbohydrate binding properties. The increasing use of lectins in chemical and biological aspects has prompted for their purification. By definition, lectins are carbohydrate-binding proteins of non-immune origin, which agglutinate cells and/or precipitate glycoconjugates/polysaccharides. The interaction of lectin with carbohydrates is very specific, as the enzyme-substrate, or antigen-antibody interactions. Lectin induced agglutination is exhibited by a special sugar that distinguishes them from mammalian antigens. Lectins can induce bacterial cell agglutination thus inhibiting their growth. This characteristic may be useful as an effective tool in the identification of pathogens and to control infections caused by them.

Lectins have been classified into several families based on their sugar specificity and extensive homology among its members. Based on lectin-carbohydrate interaction chemistry they are classified into mannose-specific, galactose-specific, N-acetyl glucosamine-specific, L-fucose-specific and sialic acid specific.

Lectins comprise one of the most abundant classes of proteins in plants. Most storage organs such as seeds contain lectins. Cajanus cajan (L.) Millsp. (Pigeon pea) is one of the oldest food crops and ranks fifth in importance among edible legumes of the world. It is important in human nutrition as a rich source of dietary protein. A lectin present in roots of Cajanus cajan seedlings was isolated and purified by affinity chromatography. Sugar specificity assayed by hemagglutination-inhibition activity, indicated that lectin belongs to glucose/mannose specific group. The root lectin was found to be mannose specific and was reconfirmed by specific elution of different days’ sample from mannose agarose matrix. Lectin (total amout of eluted protein) from different days soil sample showed a maximum amount in 10-day-old sample and also the maximum interaction with goat IgM indicating the highest lectin content. For further studies, the lectin has been isolated from
the roots of 10-day *Cajanus cajan* seedlings and purified on mannose-CL agarose column by affinity chromatography. Lectin was found to be a dimer of 18.5 KDa subunit as revealed by SDS-PAGE. Secondary structure of *Cajanus cajan* root lectin as studied by circular dichroism was found to be a typical β-pleated sheet structure. The interaction of purified root lectin with *Cajanus cajan* specific rhizobial lipopolysaccharide and its inhibition by specific and non specific sugars was demonstrated by fluorescence and circular dichroism.

Concanavalin A is a lectin from jack bean, and is a well-studied protein because of its molecular structure and many medical applications. A systematic investigation of the effect of polyethylene glycol (PEG) 200 and 400 on the solution conformation of concanavalin A (con A) was made using circular dichroism (CD), tryptophan fluorescence, 1-anilino-8-naphthalenesulfonic acid (ANS) binding, and size-exclusion chromatography. Far-UV CD spectra of con A at 30%(v/v) PEGs show the retention of ordered secondary structure as compared to 70%(v/v) PEGs. Near-UV CD spectra showed the retention of native-like spectral features in the presence of 30%(v/v) PEGs. Intrinsic tryptophan fluorescence studies indicate a change in the environment of tryptophan residues on the addition of PEG. ANS binding was maximum at 30%(v/v) PEGs suggesting the compact “molten-globule”-like state with enhanced exposure of hydrophobic surface area. Size-exclusion chromatography indicates an intermediate hydrodynamic size at 30%(v/v) PEGs. Guanidine hydrochloride (GdnHCl) denaturation of these states was a single-step, two-state transition. To study the minimum structural requirement for specific binding, the different PEGs induced states were examined for their interaction with ligand by turbidity measurements. The C$_{50}$ value was less in PEG 400 suggesting the more inhibitory ability of PEG 400. The C$_{50}$ value of PEGs was highest for dextran followed by glycogen, ovalbumin, and ovamucoid. From percentage inhibition of con A–ligands at 30%(v/v) PEGs, maximum inhibition was in ovalbumin followed by ovamucoid, glycogen, and dextran. To summarize: con A at 30%(v/v) PEGs exists as compact intermediate with molten-globule-like characteristics, viz., enhanced hydrophobic surface area, retention of compact secondary as well as tertiary structure, and a considerable degree of carbohydrate binding activity. These results has significant implications on the molten globule state during the folding pathway(s) of proteins in
general and quaternary association in the legume lectin in particular, where precise topology is required for their biological activities.

*Clitoria ternatea* or Butterfly pea commonly known as Shankupushpam, is widely used in traditional Indian systems of medicine as a brain tonic and is believed to promote memory and intelligence. A lectin present in seeds of *Clitoria ternatea* was isolated and purified for the first time by the combination of acetic acid precipitation, salt fractination and affinity chromatography on either fetuin or asialofetuin CL sepharose. The lectin agglutinated trypsin-treated human B erythrocytes. Sugar specificity assay indicated that lectin belongs to Gal/Gal NAc-specific group. Gel filtration, SDS-polyacrylamide gel electrophoresis indicated that the native lectin, designated *C. ternatea* agglutinin (CTA), is composed of two identical subunits of molecular weight 34.7 Kd associated by non covalent bonds. The N-terminal sequence of CTA shared the homology with *Glycine max* and *Pisum sativum*. Complete sequence was also found to be homologous to S-64 protein of *Glycine max*, suggesting that CTA exhibits both haemagglutination as well as sugar uptake activity. The two activities are probably associated with separate loci on the same protein. Secondary structure of *C. ternatea* agglutinin as studied by circular dichroism (CD) was found to be predominately β-pleated sheet structure. CD conformational studies revealed changes in the near as well as far–ultra violet spectrum at the extremes of pH. The reaction of lectin with glycoprotein was affected by pH changes. CD conformational and increased activity with fetuin in the presence of metal ions suggests that CTA is metal ion requiring lectin. The carbohydrate binding specificity of the lectin was investigated by quantitative turbidity measurements, hemagglutination inhibition, and percent inhibition assays. Based on the results obtained by these assays, we conclude that although the *C. ternatea* agglutinin binds β-D-galactosides, it has an extended carbohydrate-combining site that exhibits highest specificity and affinity towards non-reducing terminal Neu5Aco2,6Galβ1,4Glc. The specificity of the lectin for α2,6-linked sialic acid renders this lectin a valuable tool for glycobiology studies in biomedical and cancer research.