J. Biosci., Vol. 5, *Supplement* 1, December 1983, pp. 131-135. © Printed in India.

Plant lectins specific for N-acetyl-β-D-galactosamine

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Abstract. N-Acetyl-D-galactosamine in β-linkage being ubiquitous in cell surface glycoproteins, their interaction with lectins specific for this sugar moiety may be a significant event in cell adhesion phenomena. This article discusses the common β-N-acetyl galactosamine-specific lectins, with particular stress on the lectin from winged beans (*Psophocarpus tetragonolobus*).

Keywords. β-N-Acetyl-D-galactosamine; blood group A; winged bean.

Introduction

In recent years, lectins have been recognised as useful probes for structural investigations of polysaccharides and complex carbohydrates on cell surfaces. Some lectins are highly specific for certain blood groups and thus may be used for blood group typing. Certain lectins differentiate malignant from normal cells (Sharon and Lis, 1972). Various hypotheses have been advanced regarding the natural physiological function of lectins in plants and animals that contain them. Unequivocal proof is now available for the suggestion that lectins of leguminous plants mediate the attachment of nitrogen fixing bacteria to their root nodules (Bohlool and Schmidt, 1974). Empirical findings suggest that animal lectins may be involved in cell-cell adhesion and differentiation (Rosen *et al.,* 1973; Yamada *et al.,* 1975). The proposed role for lectins in sugar transport is yet to be demonstrated (Brisk and Chrispeals, 1972). Any of these functions however, involves the interaction of lectins with glycoconjugates. Since the latter often contain N-acylated glycosamines investigations on lectins specific for these sugar moieties would be fruitful.

Lectins that specifically bind N-acetyl-D-galactosamine are particularly important since the latter is the determinant sugar moiety in blood group A substance on the erythrocyte surface. The present paper is a discussion on the widely used N-acetyl-D-galactosamine binding lectins.

Soybean (*Glycine max*) lectin

Soybean lectin (SBA) was originally purified by Liener and Pallansch (1952). Later Sharon and his co-workers have purified the same lectin by affinity chromato graphy on a column of 6-aminocaproyl-β-D-galactosylamine coupled to Sepharose (Gordon *et al.,* 1972). They have studied extensively the physicochemical

Abbreviations used: SBA, Soybean lectin; M_r, molecular weight; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; PHA, phytohaemagglutinin; WBA, winged bean agglutinin.

properties of the lectin. The molecular weight (M_r) was found to be 110,000 dalton consisting of four identical subunits of 30,000 ± 500. The protein was therefore a tetramer (Lis and Sharon, 1973). It contains 4.5% D-mannose and 1.2% N-acetyl-D-glucosamine (Lis *et al.,* 1966). The carbohydrate linkage of SBA was through asparagine moiety to N-acetyl-β-D-glucosamine moiety. The carbohydrate-peptide linking group was identified as. (Man) $_{4–5}$ GlcNAc (Man) $_{5–4}$ (GlcNAc) $_{1–2}$-ASn. All the mannose residues are α-linked and the N-acetyl-D-glucosamine residues are β-linked. The soybean oil meal was found to contain three minor isolectins which are separable on DEAE-cellulose columns (Yamada *et al.*, 1975).

The agglutination of erythrocytes or malignant tissue culture cells by SBA is strongly and specifically inhibited by GalNAc (0.05-0.1 μmol/ml) or by disaccharide with GalNAc at their non-reducing end and to a lesser degree by Gal (Lis *et al.,* 1970). SBA contains two identical binding sites for GalNAc per 120,000 dalton with $Ka = 3.0 \times l0^4$/mol.

Lima bean (*Phaseolus lunatus*) lectin

Lima bean lectin was the first plant haemagglutinin shown to exhibit blood group specificity (Boyd and Reguera, 1949). The agglutinating activity resided in two isolectins with the same aminoacid and carbohydrate composition, but the M_r of one (component II, 110,000-138,000) was twice that of the other isolectin (component III, 195,000-269,000) (Gailbraith and Goldstein, 1972a). On sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with β-mercaptoethanol, both components yielded identical subunits of M_r about 60,000 dalton. It has been suggested that lima bean isolectins are tetramer and dimer of 60,000 dalton disulphide-containing subunits.

These lectins are also glycoproteins, each containing 6–7 mol mannose, 2 mol glucosamine, 1 mol fucose and pentose per 31,000 *g* protein (Gailbraith and Goldstein, 1972a). They also contain Mn^{2+} and Ca^{2+} and the amount of Mn^{2+} is 0.7–11 mol/mol component III and 1.2 mol/mol component II (Gailbraith and Goldstein, 1972b). Lima bean lectins are specific for type A human erythrocytes and N-acetyl-D-galactosamine is specific and reversible inhibitor of the lectins.

Red kidney bean (*Phaseolus vulgaris*) lectin

The term phytohaemagglutinin (PHA) usually refers to a crude preparation from red kidney bean. Crude PHA possessed erythroagglutinin, leucoagglutinin and mitogenic activities. This was the first lectin shown to be mitogenic (Nowell, 1960; Robins, 1964). Several groups have attempted to separate the various activities, but results were often contradictory and somewhat confusing (Lis and Sharon, 1973). The M_r of the lectins, range from 91,000 to 150,000 having 8-4 subunits. Even there was discrepancy in the subunit M_r ranging from 29,000 to 36,000. This confusion can only be settled by further work on the lectins. The lectins are glycolproteins containing mannose and glucosamine, while certain preparation contained fucose, xylose and arabinose, in addition to the above mentioned carbohydrates (Ohtani *et al.,* 1980).

Vicia cracca lectin

Vicia cracca lectin had been isolated by Ruediger (1977) and Karhi and Gahmberg (1980) independently. The methods of affinity chromatography used by the two laboratories are different. Ruediger had used N-acetyl-D-galactosamine coupled to CNBr-activated Sepharose with spacer, while the latter author had used immobilised porcine gastric A/H substance. The physicochemical and biological properties of the isolated lectins were identical. The native lectins had a M_r of 100,000 with a subunit M_r of 29,000. Ruediger (1977) had isolated two isolectins by affinity chromatography. Karhi and Gahmberg (1980) had not mentioned any isolectins. They had shown that the lectin was a glycoprotein contaning 5% carbohydrate with mannose and N-acetyl-glucosamine as the major carbohydrates. It was also shown that the protein contained no sulphur aminoacids and a relatively higher content of aspartic acid. The lectins isolated from both the laboratories were blood group A specific.

Horse gram (*Dolichos biflorus*) lectin

This lectin had been purified to homogeneity on polyleucyl hog AH substance column. It is a glycoprotein with a M_r of 141,000 and had about four subunits. It contained no cysteine. It precipitated blood group A_1 and A_2 substances and other polysaccharides having terminal non-reducing N-acetyl-D-galactosamine residues. Etzler and Kabat (1970) and Etzler (1972) have shown the importance of N-acetyl group in C-2 and the stereochemistry of the hydroxyl on C-4. Recently Etzler and Borrebaeck (1980) have shown the presence of a cross-reacting immunomaterial in the extracts of stem and leaves of the *Dolichos biflorus* plant. The antibody was prepared against the homogeneous lectin from the seeds (Etzler and Borrebaeck,1980).

Other GalNAc binding proteins of plant origin had been purified from the seeds of *Wistaria floribunda* by Osawa and Toyoshima (1972) as well as from the seeds of *Sophora japonica* by Poretz (Poretz, 1972). These two lectins are also inhibited by other sugars.

Winged bean (*Psophocarpus tetragonolobus*) lectin

In the course of a search for carbohydrate-binding proteins from leguminous seeds, an N-acetyl-D-galactosamine-binding protein from winged bean was purified to homogeneity in this laboratory (Appukuttan and Basu, 1981). The seeds were obtained from locally cultivated beans. The winged bean agglutinin (WBA) was purified by affinity chromatography on N-caproylated galactosamine coupled to Sepharose. Unlike the N-acetyl-D-galactosamine binding lectins described above WBA is not a glycoprotein. Molecular weight determined by gel filtration on Biogel-P 100 and on SDS-PAGE in the presence of 2-mercaptoethanol gave values of 41,000 and 35,000 respectively (figure 1). This lectin like wheat germ agglutinin is a single polypeptide unit containing at least two, binding sites for sugar so that it can behave as an agglutinin.

This lectin also binds to galactose though to a lesser extent than N-acetyl-D-galactosamine. Table 1 gives the inhibition capacities of various sugars in agglutination by the lectin.

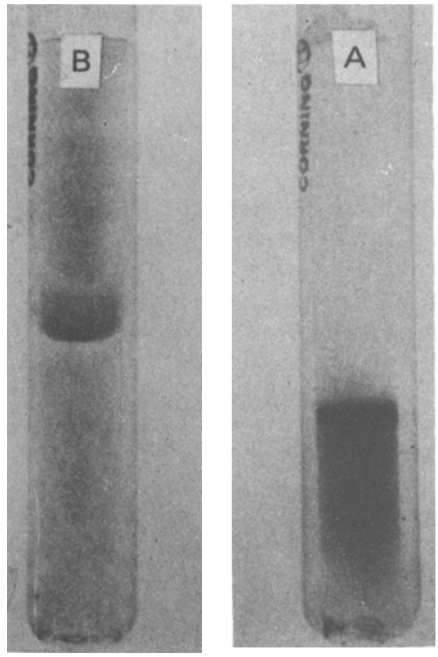

Figure 1. A. Polyacrylamide gel electrophoresis of purified winged bean agglutinin at alkaline pH (8.3). Twenty-five µg was used per tube. **B.** SDS-PAGE of the same protein Details are given in methods. Twenty-five µg protein was used per tube.

Table 1. Inhibition capacities of common sugars on agglutination of erythrocytes by winged bean agglutinin.

Sugar	Minimum concentration (mM) required to inhibit twice the haemagglutinating amount of lectin
Glucose	100
Galactose	12.5
Mannose	N.I.
N-Acetyl-D-glucosamine	100
N-Acetyl-D-galactosamine	1.56
Lactose	6.25
Fucose	N.I.
Galactosamine	N.I.
Ribose	100
D-Fructose	N.I.
α-Methylglucoside	100

Aliquots of 0.2 ml in PBS containing 2 µg protein (double the minimum amount of protein for haemagglutination)-with or without serial dilutions of the sugars were incubated for an hour at 25° C and 0.05 ml of 5% suspension of erythrocytes in PBS added. Agglutination was noted after 2 h with occasional shaking.

N.I.– No inhibitory effect even upto 200 mM concentration.

A notable feature of the general affinity chromatographic method of isolation of the N-acetyl-D-galactosamine binding lectins is that the N-caproyl-D-galactosamine group attached to the matrix also binds the lectin. Thus the acyl group need not necessarily be acetyl.

Acknowledgement

This project was financed by Grant No. ll (28)/79-SERC from Department of Science and Technology, New Delhi.

References

Appukuttan, P. S. and Basu, D. (1981) *Anal. Biochem.,* **113,** 253.
Bohlool, B. B. and Schmidt, E. L. (1974) *Science,* **185,** 269.
Boyd, W. C. and Reguera, R. M. (1949) *J. Immunol.,* **62,** 333.
Brisk, M. M. and Chrispeals, M. J. (1972) *Biochim. Biophys. Acta,* **257,** 421.
Etzler, M. E. and Kabat, E. A. (1970) *Biochemistry,* **9,** 869.
Etzler, M. E. (1972) *Meth. Enzymol.,* **28,** 340.
Etzler, M. E. and Borrebaeck, C. (1980) *Biochem. Biophys. Res. Commun.,* **96,** 92.
Gailbraith, W. and Goldstein, I. J. (1972a) *Biochemistry,* **11,** 3976.
Gailbraith, W. and Goldstein, I. J. (1972b) *Meth. Enzymol.,* **28,** 318.
Gordon, J. A., Blumberg, S., Lis, H. and Sharon, N. (1972) *FEBS Lett.,* **24,** 193.
Karhi, K. K. and Gahmberg, C. G. (1980) *Biochim. Biophys. Acta,* **622,** 337.
Liener, I. E. and Pallansch, M. J. (1952) *J. Biol. Chem.,* **197,** 29.
Lis, H., Sharon, N. and Katchalski, E. (1966) *J. Biol. Chem.,* **241,** 684.
Lis, H., Sela, B. A., Sachs, L. and Sharon, N. (1970) *Biochim. Biophys. Acta,* **211,** 582.
Lis, H. and Sharon, N. (1973) *Ann. Rev. Biochem.,* **42,** 541.
Nowell, P. C. (1960) *Cancer Res.,* **20,** 462.
Ohtani, K., Shibata, S. and Misaki, A. (1980) *J. Biochem.,* **87,** 407.
Osawa, T. and Toyoshima, S. (1972) *Meth. Enzymol.,* **28,** 328.
Poretz, R. D. (1972) *Meth. Enzymol.,* **28,** 349.
Robins, J. H. (1964) *Science,* **146,** 1648.
Rosen, S. D., Kafka, J., Simpson, D. L. and Barondes, S. H. (1973) *Proc. Natl. Acad. Sci. USA,* **70,** 2554.
Ruediger, H. (1977) *Eur. J. Biochem.,* **72,** 317.
Sharon, N. and Lis, H. (1972) *Science,* **177,** 949.
Yamada, K. M., Yamada, S. S. and Pastan, I. (1975) *Proc. Natl. Acad. Sci. USA,* **72,** 3158.