Carbohydrate Polymers **10** (1989) 281–288

Review Paper

Rice Lectin: Physico-chemical and Carbohydrate-Binding Properties

Indravathamma Poola

Department of Biochemistry and Molecular Biology, SUNY Health Science Center, Syracuse, New York 13210, USA

(Received 10 August 1988; accepted 27 October 1988)

ABSTRACT

Rice seeds contain a 2-acetamido-2-deoxy-D-glucose-specific lectin. It has an M_r of 36 000 and is composed of two identical, non-covalently bound subunits of M_r 18 000. Each subunit consists of two disulfide-linked polypeptide chains of M_{rs} 10 000 and 8000. The lectin activity is highly stable to several chemical denaturants and heat treatment. The lectin interacts with glycoproteins, which have either clustered O-linked oligosaccharides or N-linked oligosaccharides. The N-linked glycoproteins include high D-mannose, hybrid and complex biantennary structures.

INTRODUCTION

Lectins are carbohydrate-binding proteins which agglutinate cells and precipitate glycoconjugates or polysaccharides (Goldstein *et al.*, 1980). By virtue of their sugar-binding properties, lectins have found high applicability in several branches of biological research in detecting and studying carbohydrates in solution and on cell surfaces. They are found in several plant and animal sources. One important class of plant materials in which lectins are found is leguminous seeds. Several other food items also contain lectins. Plant lectins are toxic in their native form if taken orally or injected peritoneally. The toxicity of lectins is due to their ability to bind to specific carbohydrate receptor sites on the intestinal mucosal cells and interference with the absorption of nutrients across the intestinal wall (Liener, 1986). In view of these toxic effects, it

Carbohydrate Polymers 0144-8617/89/$03.50

becomes very important to understand the precise mode of the interaction of lectins found in common food materials with cell-surface glycoconjugates. Rice is one of the most important food materials as it constitutes half the diet for 1·6 billion people, and another 100 million rely on it for between a fourth and half of their diet (Swaminathan, 1984). Rice contains a 2-acetamido-2-deoxy-D-glucose-binding lectin. The purification, physico-chemical and carbohydrate-binding properties are described in this review.

Takahashi *et al.* (1973) first reported the presence of a hemagglutinin in rice seeds. They purified the lectin by conventional chromatographic methods and partially characterized it as a glycoprotein with an M_r of 10 000. However, they did not study its sugar-binding characteristics. Poola and Seshadri (1979) were the first to discover the specificity of rice lectin for 2-acetamido-2-deoxy-D-glucose. Consequently, they purified the lectin from rice flour on a 2-acetamido-2-deoxy-D-glucose–Sepharose affinity column (Poola & Seshadri, 1980). High yields of the lectin were obtained by purification on a 4-aminobenzyl 1-thio-2-acetamido-2-deoxy-β-D-glucopyranoside-succinyl-amino-hexylaminyl–Sepharose 4B affinity column (Poola *et al.*, 1986). A 2-acetamido-2-deoxy-D-glucose-specific lectin was also purified from rice bran on an ovomucoid–Sepharose column (Tsuda, 1979). The bran and the flour lectin have a similar molecular weight of 36 000, and are composed of two identical non-covalently linked subunits of M_r 19 000. Each subunit is composed of two disulfide-linked polypeptide chains of M_{rs} 10 000 and 8000 (Tsuda, 1979; Poola *et al.*, 1986). Similar molecular properties were also reported by Peumans and Stinissen (1982), although they stated that the polypeptide chains (M_r 10 000 and 8000) were the proteolytic cleavage products of 18 000 molecular weight species rather than disulfide-linked chains (Peumans & Stinissen, 1982). Somewhat contradictory values were reported by Tabary *et al.* (1987). According to these workers, rice lectin has an M_r of 36 000 and is composed of two non-identical, disulfide-linked subunits of M_{rs} 19 000 and 15 000.

Amino-acid analysis of the rice lectin indicated a high proportion of glycine, glutamic acid and half cystinyl residues (Tsuda, 1979; Poola *et al.*, 1986). Poola *et al.* have reported about 50 half cystines per 36 000, whereas 44 were reported for rice bran lectin by Tsuda. All of the half cystinyl residues seem to be engaged in disulfide linkages, as no free sulfhydryl residues were detected in the purified lectin (Poola *et al.*, 1986). The controversial values reported for the subunit molecular weights by Tabary *et al.* (1987) may be due to the high proportion of disulfide linkages in the lectin molecule. It is very well known that the proteins which have a high content of inter- and intra-disulfide linkages will give anomalous apparent molecular weight values upon SDS-PAGE,

if not reduced completely (Allore & Barber, 1984). Rice lectin, if exposed to acid pH, exhibits high resistance to disulfide cleavage by reducing agents (Tsuda, 1979; Poola *et al.*, 1986). Tabary *et al.* (1987) have purified the lectin by acid extraction (pH 1·3) and affinity chromatography on an agarose-4-aminophenyl 2-acetamido-2-deoxy-β-D-glucose column. The apparent molecular weight values (19 000 and 15 000) reported by them seem to be due to the resistance of their acid-extracted lectin to reducing agents of disulfide cleavage, and hence the anomalous migration of protein bands upon SDS–PAGE.

Rice lectin has no covalently bound carbohydrate. Several reports about the presence of various sugars were proved to be contaminants (Takahashi *et al.*, 1973; Poola & Seshadri, 1979, 1980). However, it was observed that if the extraction and purification of the lectin involves buffers below pH 5·0 such as described by Poola and Seshadri (1980), a small molecular weight (M_r 10 000–11 000), pentose-containing glycoprotein co-purifies with rice lectin. The carbohydrate structure of the pentose-containing glycoprotein appears to be an L-arabino-D-xylan (Poola & Seshadri, unpublished).

Rice lectin offers high resistance to heat denaturation and pH changes. Poola and Seshadri have heated the lectin solution in phosphate-buffered saline at various temperatures and for various lengths of time, and found that the erythroagglutinating activity is stable for 2 h at 75°C but sharply loses activity after heating for 30 min at 80°C (Poola & Seshadri, 1984). Peumans and Stinissen (1982) have also made similar observations. The agglutinating activity is also stable after exposing the lectin in the pH range 2–12 (Poola & Seshadri, 1984). Exposure of the lectin to denaturants such as 8 M urea, 6 M guanidine hydrochloride and 0·1% SDS does not destroy the lectin activity. It is also highly resistant to proteolytic enzymes such as trypsin, pepsin and chymotrypsin. The high resistance of the lectin to the denaturing conditions is presumably due to its compact stable conformation, offered by several disulfide linkages. Wheatgerm agglutinin, with which rice lectin shares certain physicochemical and carbohydrate-binding properties, also contains several disulfide bridges and exhibits high resistance to several denaturing conditions (Wright *et al.*, 1984).

CARBOHYDRATE-BINDING PROPERTIES OF RICE LECTIN

The carbohydrate-binding specificity of rice lectin was studied extensively by Poola and co-workers (Poola & Seshadri, 1980; Poola *et al.*, 1986; Poola & Kella, 1986; Poola & Narasimhan, 1988). By a hemagglutination-inhibition method, it was found (Poola *et al.*, 1986) that

methyl 2-acetamido-2-deoxy-β-D-glucopyranoside was the best monosaccharide inhibitor (10 times more inhibitory than 2-acetamido-2-deoxy-D-glucose) of rice lectin-induced hemagglutination. D-Arabinose, D-xylose, D-lyxose, L-fucose, D-glucose, D-galactose and D-fructose were all found to be non-inhibitory even at 200 mM concentrations. 2-Acetamido-2-deoxy-D-galactose was a very poor inhibitor (about 20–30 times less inhibitory than 2-acetamido-2-deoxy-D-glucose) and 2-amino-2-deoxy-D-glucose was a non-inhibitor. The poor inhibitory power of 2-acetamido-2-deoxy-D-galactose and non-inhibitory effect of 2-amino-2-deoxy-D-glucose demonstrates the necessity of an equatorial hydroxyl group at C-4 and an acetamido group in the D-*glycero* configuration at C-2 for binding to rice lectin, as in the case of wheatgerm agglutinin and GS II lectins (Goldstein *et al.*, 1975; Iyer *et al.*, 1976). The rice lectin shows higher affinity for chitin oligomers than for monosaccharide. Thus the chitobiose and triose were about 500 and 5000 times more inhibitory than the monosaccharide (Poola *et al.*, 1986). Based on these observations Poola *et al.* have postulated that rice lectin has an extended binding site which may be complementary to a sequence of (1→4)-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl units. Of the several other saccharides tested, only *N*-acetylneuraminyl (2→3)- and (2→6)-β-D-galactopyranosyl (1→4)-D-glucopyranose showed some affinity to rice lectin (Poola *et al.*, 1986), although the *N*-acetylneuraminic acid was a non-inhibitor. Lactose, sucrose, maltose and melibiose were non-inhibitory even at 200 mM concentrations. By equilibrium dialysis and fluorescence quenching titrations, Poola and Kella (1986) have measured the number of sugar-binding sites on rice lectin. They obtained four identical and independent binding sites per mol protein (M_r 36 000), with an association constant of $4{\cdot}82 \times 10^4$ M^{-1} at 25°C. Values of $1{\cdot}16 \times 10^4$ M^{-1} (Tsuda, 1979) and $1{\cdot}3 \times 10^3$ M^{-1} (Tabary & Frenoy, 1985) were also reported. Binding experiments by fluorescence-quenching titrations also suggested that rice lectin has an extended carbohydrate-binding site. Studies on the thermodynamics of sugar binding to rice lectin showed that binding constants decreased with increasing temperature, indicating that the binding of sugar to the lectin is exothermic in nature (Poola & Kella, 1986).

Having understood the interaction of rice lectin with monosaccharides and linear oligosaccharides, Poola *et al.* (1986) extended their studies to understand the interaction of rice lectin with glycoproteins. They have tested the binding capacity of several mucin-type glycoproteins, which have only serine/threonine-linked oligosaccharides, asparagine-linked oligosaccharides and those glycoproteins which have both. They studied the above by two techniques: inhibition of hemag-

glutination and binding affinity of glycoproteins to immobilized lectin. Glycophorin, a major sialoglycoprotein of human erythrocyte membrane, which has ten clustered O-glycosidically linked oligosaccharides, showed very high affinity. The binding affinity was lost upon sialidase treatment. Based on these observations Poola *et al.* (1986) have concluded that rice lectin interacts with the mucin-type glycoproteins through several terminal neuraminic acid residues present in clustered O-linked oligosaccharides. In support of this conclusion, salivary glycoprotein, which has several O-linked neuraminyl-oligosaccharides was found to be a potent inhibitor of rice lectin-induced hemagglutination. After studying the interaction with several other O-linked glycoproteins Poola *et al.* have proposed that a glycoprotein should have at least three O-linked neuraminyl-oligosaccharides, as in the case of fetuin, for efficient binding. Although the interaction of rice lectin with mucin-type glycoproteins were clearly understood by the above studies, the nature of interaction with N-linked glycoproteins could not be drawn.

To gain more insight into these points, Poola and Narasimhan (1988) have studied the interaction of several structurally characterized asparagine-linked oligosaccharides to immobilized rice lectin (Poola & Narasimhan, 1988). By their extensive studies it was found that rice lectin binds asparagine-linked oligosaccharides through the core chitobiosyl moiety, the (1→4)-linked β-D-mannopyranosyl residue slightly contributes to the binding capacity, but the (1→3)-linked core α-D-mannopyranosyl residue greatly enhances the binding strength and is a very important residue for binding of rice lectin to asparagine-linked oligosaccharides. A core (1→6)-linked α-D-mannopyranosyl residue has a slight inhibitory effect. Although a core (1→6)-linked α-L-fucopyranonyl residue does not have any effect, 2-acetamido-2-deoxy-D-glucosyl-asparagine linkage contributes slightly to the binding. The outer D-mannopyranosyl residues do not interact with the lectin. Poola and Narasimhan have further studied the binding characteristics of complex bisected and non-bisected biantennary and triantennary asparagine-linked oligosaccharides. The outer (1→2)-linked 2-acetamido-2-deoxy-β-D-glucose and (1→4)-linked β-D-galactopyranosyl residues of the complex biantennary structures do not contribute to the binding. However, neuraminic acid in the terminal position decreases the binding strengths of complex biantennary asparagine-linked oligosaccharides, probably by interfering with the 'docking' of the glycopeptide by steric or charge–charge interactions at the binding site.

The effect of a (1→4)-linked 2-acetamido-2-deoxy-β-D-glucose linked to (1→4)-linked β-D-mannopyranose (a bisecting 2-acetamido-2-deoxy-D-glucose) was also thoroughly investigated, as several lectins are

affected by this residue. Interestingly, this lectin showed a very great affinity for this residue. Complex biantennary and the hybrid types which have a bisecting 2-acetamido-2-deoxy-D-glucose bind very tightly to the lectin. Studies on the triantennary structures revealed that substitution of C-2 and C-4 of the core (1 → 3)-linked α-D-mannopyranose with 2-acetamido-2-deoxy-D-glucose completely abolished interaction with rice lectin. Several lectins, such as concanavalin A, pea and lentil lectins, and L-PHA, share this property with rice lectin (Narasimhan *et al.*, 1979; Kornfeld *et al.*, 1981; Cummings & Kornfeld, 1982). Based on the above studies the best complementary N-linked oligosaccharide structure for rice lectin is proposed in Fig. 1.

OTHER PROPERTIES OF RICE LECTIN

Rice lectin agglutinates rabbit, rat, hamster, guinea-pig and monkey erythrocytes. It agglutinates human A, B, and O group erythrocytes equally well. Normal and transformed human leukocytes and HeLa cells were also agglutinated by rice lectin (Poola, 1982; Poola *et al.*, 1986). It binds to the bacteria which are found in the rhizosphere of rice (Tabary *et al.*, 1984). Poola *et al.* (1986) have observed that rice lectin stimulated the incorporation of [^{3}H]thymidine by human peripheral lymphocytes. Similar results were observed by Tabary *et al.* (1987) although neither blast formation nor lymphocyte proliferation were reported.

APPLICATION OF RICE LECTIN IN CARBOHYDRATE RESEARCH

As described earlier, rice lectin binds glycoproteins which have either O-linked oligosaccharides or N-linked oligosaccharides. The N-linked glycoproteins include a high D-mannosyl type, a complex biantennary which can or cannot have a bisecting 2-acetamido-2-deoxy-D-glucose

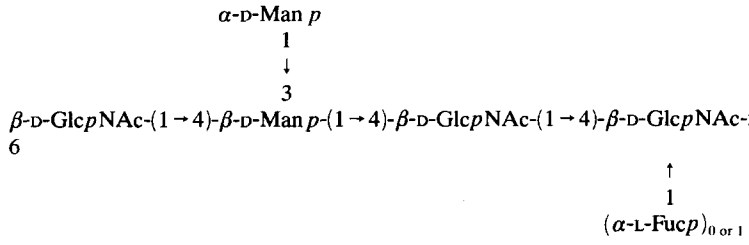

Fig. 1. Proposed N-linked oligosaccharide structure for rice lectin.

interacting with rice lectin. The presence of asparagine-linked oligosaccharides are generally detected and isolated using concanavalin A, and the O-linked glycoproteins by wheatgerm agglutinin.

By virtue of its high affinity for both O-linked and N-linked oligosaccharides, rice lectin could be applied to detect the presence of a carbohydrate on a biopolymer instead of probing with wheatgerm agglutinin and concanavalin A.

Poola and Narasimhan (1988) have shown that the immobilized rice lectin column could be applied to separate the asparagine-linked oligosaccharides into eight separate fractions, in combination with an immobolized concanavalin A column. The differential affinity of rice lectin–Sepharose column for neuraminic acid and a bisecting 2-acetamido-2-deoxy-D-glucose could also be exploited to study the differences in the glycosylation pattern in normal and transformed cells. Finally, the rice lectin column could be applied to study the microheterogeneity of a glycoprotein in its carbohydrate moieties.

FURTHER RESEARCH

Although the carbohydrate-binding characteristics of rice lectin are understood in detail, the chemical nature of rice lectin needs to be studied to understand the primary and three-dimensional structure of this important plant lectin. This would enable us to understand the structure–function relationships and its structural homologies with other graminaceous lectins.

Only a few varieties of rice strains seem to contain the lectin activity (Poola & Seshadri, 1980). In view of this, it is important to estimate the lectin content of a particular rice variety and its stability to various processing steps and in fully processed and semi-processed foods. Finally, the toxic effects of rice lectin, if any, need to be evaluated.

REFERENCES

Allore, R. J. & Barber, B. H. (1984). *Anal. Biochem.*, **137**, 523.
Cummings, R. D. & Kornfeld, S. (1982). *J. Biol. Chem.*, **257**, 11230.
Goldstein, I. J., Hammarstrom, S. & Sundblad, G. (1975). *Biochim. Biophys. Acta.*, **405**, 53.
Goldstein, I. J., Hughes, R. C., Monsigny, M., Osawa, T. & Sharon, N. (1980). *Nature*, **285**, 66.
Iyer, P. N. S., Wilkins, K. D. & Goldstein, I. J. (1976). *Arch. Biochem. Biophys.*, **177**, 330.

Kornfeld, K., Reitman, M. L. & Kornfeld, R. (1981). *J. Biol. Chem.*, **256**, 6633.
Liener, I. E. (1986). In *The Lectins: Properties, Functions and Applications in Biology and Medicine*, eds I. E. Liener, N. Sharon & I. J. Goldstein. Academic Press, New York, p. 527.
Narasimhan, S., Wilson, J. R., Martin, E. & Schachter, H. (1979). *Can. J. Biochem.*, **57**, 83.
Peumans, W. J. & Stinissen, H. M. (1982). *Arch. Intern. Physiol. Biochim.*, **90**, B210.
Poola, I. (1982). PhD thesis, The University of Mysore, Mysore.
Poola, I. & Kella, N. K. D. (1986). *Biochim. Biophys. Acta*, **882**, 12.
Poola, I. & Narasimhan, S. (1988). *Biochem. J.*, **250**, 117.
Poola, I. & Seshadri, H. S. (1979). *Indian J. Biochem. Biophys.*, **16** (Suppl) Abstr. 18.
Poola, I. & Seshadri, H. S. (1980). *J. Biosci.*, **2**, 29.
Poola, I. & Seshadri, H. S. (1984). *Indian J. Biochem. Biophys.*, **21**, 137.
Poola, I., Seshadri, H. S. & Bhavanandan, V. P. (1986). *Carbohydr. Res.*, **146**, 205.
Swaminathan, M. S. (1984). *Sci. Am.*, **250**(1), 80.
Tabary, F. & Frenoy, J.-P. (1985). *Biochem. J.*, **229**, 687.
Tabary, F., Balandreau, J. & Bourrillon, R. (1984). *Biochem. Biophys. Res. Commun.*, **119**, 549.
Tabary, F., Font, J. & Bourrillon, R. (1987). *Arch. Biochem. Biophys.*, **259**, 79.
Tsuda, M. (1979). *J. Biochem.*, **86**, 1451.
Takahashi, T., Yamada, N., Iwamoto, K., Shimabayashi, Y. & Izutsu, K. (1973). *Agric. Biol. Chem.*, **37**, 29.
Wright, C. S., Gavilanes, F. & Peterson, D. L. (1984). *Biochemistry*, **23**, 280.