BINDING OF PEA LECTINS TO A GLYCAN TYPE POLYSACCHARIDE IN THE CELL WALLS OF *RHIZOBIUM LEGUMINOSARUM*

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1. Introduction

The polysaccharides of the symbiotic bacterium *Rhizobium* have been of considerable interest in recent years because of a possible role in the determination of host specificity.

At first attention has been focused on the exopolysaccharides (XPS) because of reports on the induction of polygalacturonidase in the host root [1] by rhizobial XPS.

No relation, however, between XPS composition and host specificity could be established [2–4] and the interest subsided until the appearance of reports about the specific binding of lectins, plant proteins with haemagglutinating activity, onto the *Rhizobium* outer surface [5,6].

Lectins have been reported to bind to either acidic polysaccharide of capsular origin [7] or lipopolysaccharide specifically of the *Rhizobium* species infective on the plant from which the lectins were isolated [8].

We report here the presence of a new type of polysaccharide in rhizobial lipopolysaccharides after isolation of the latter from bacterial cell wall, chemical characteristics and the interaction with lectins isolated from pea seeds.

2. Material and methods

Lipopolysaccharide (LPS) was isolated from *Rhizobium leguminosarum* A 171 by hot phenol extraction [9] of cell walls isolated as described previously [10]. The LPS was made accessible to Sephadex G-50 chromatography by splitting of the lipid A part in 1 N acetic acid at 100°C for 1.5 h. The lipid A was removed by centrifugation and the remaining polysaccharide (PS) was freeze dried. 20 mg of PS was fractionated on a column (2.6 × 60 cm) of Sephadex G-50 (medium grade, Pharmacia) and eluted with 0.1% $(NH_4)_2CO_3$. Fractions were tested for the presence of carbohydrate using the anthrone reagent [11]. Peaks were pooled, concentrated under vacuum and the ammonium carbonate was removed upon lyophilization. Neutral sugars were determined by gas chromatography on ECNSS/M (Applied Science USA) as their alditol derivatives [12] after hydrolysis of the samples in 1 N sulphuric acid at 100°C for 16 h.

Uronic acid was determined with the carbazol reagent using D-glucoronic acid as a standard [13]. Ketodeoxyoctonic acid (KDO) was determined by the thiobarbituric acid procedure using neuraminic acid as a standard [14].

XPS was isolated from the culture supernatant of cells grown on a mannitol salts medium [10] by precipitation by 3 vol. ethanol and deproteinised [4].

Lectins from pea seeds were isolated by affinity chromatography on Sephadex G-100 [15] and showed to be 98% pure by polyacrylamide gel electrophoresis [16].

Lectin–polysaccharide interaction was determined by ring-precipitation after subtle layering a 1% w/v solution of polysaccharide on 1% w/v lectin in Tris–HCl buffer, pH 6.5, at room temperature. *Escherichia coli* LPS, bovine serum albumin and buffer were used as blanks.

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3. Results and discussion

The neutral sugar composition of XPS is in agreement with the one described by Zevenhuizen for *R. leguminosarum PRE* (table 1) [4] while no heptose and KDO could be detected, indicating the absence of LPS. Uronic acid content was 14%.

LPS from *R. leguminosarum* could be isolated from cell walls by phenol extraction and purified by two ultracentrifugation steps after which it was free from nucleic acid or ribose as shown by ultraviolet absorption and gas chromatography. Upon ultracentrifugation, we observed the independent distribution of glucose and heptose over supernatant and pellet, indicating the presence of more than one polysaccharide component. But still considerable amounts of glucose were present in the final ribose-free LPS.

The polysaccharide isolated from the cell walls, indicated as LPS in table 1, contained after purification by ultracentrifugation fucose, mannose, galactose, glucose, heptose, KDO, aminosugars, uronic acid and a neutral sugar indicated by X. The latter was identified as a (di)deoxy sugar or methyl pentose by paper chromatography after spraying the chromatograms with piperazin-nitroprussid [17]. A study on the nature of the non-identified component, the amino sugars and the uronic acid, is in progress (Planqué and van Nierop in prep.).

Upon fractionation of PS on Sephadex G-50 three major peaks were observed (fig.1) indicated as PS I, PS II and PS III in accordance with the sequence of elution.

PS I is eluted just after the void volume and contains 87–96% glucose. Uronic acid was present at a low concentration. The different ratio of glucose to galactose and uronic acid makes it very unlikely that PS I is residual XPS.

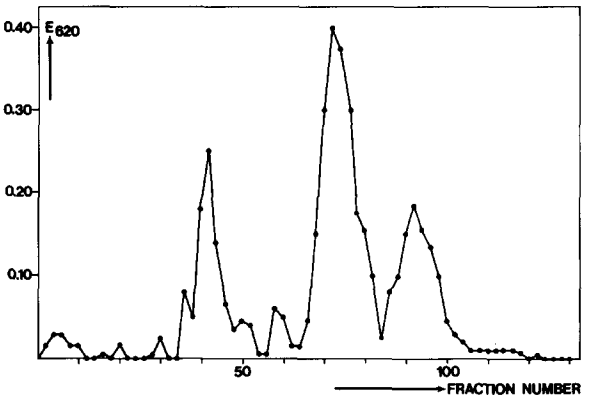

Fig.1. Elution pattern of Sephadex G-50 (60 × 2.6 cm) column loaded with 20 mg PS, as determined by anthrone procedure, fractions of 3.2 ml were collected.

The larger molecular size of PS I compared with PS II, and the absence of heptose, makes it very unlikely that PS I has been split off following degradation of LPS during the mild acid hydrolysis.

The existence of a glycan in the LPS is also supported by independent separation of glucose and heptose upon chromatography of LPS on Sephadex G-100 in the presence of 0.1% sodium dodecyl sulphate (Planqué and van Nierop in prep.).

PS II contains the sugars representative for LPS like heptose, deoxy sugars and some KDO. Heptose,

Table 1
Chemical composition of polysaccharide fractions

	(10^{-7} mol/mg)						(% dwt)	
	X	Fucose	Mannose	Galactose	Glucose	Heptose	KDO	Uronic acid
XPS	0	0	0.5	2.2	9.2	0	0	14
LPS	2.0	2.0	1.1	1.2	1.2	1.8	2.5	15
PS	1.4	1.4	0.9	1.1	2.1	1.6	2.5	16–18.3
PS I	0	0	0.8	0.3	9.9	0	[a]	0.9
PS II	4.2	3.2	0.3	0.4	0.6	3.7	0.5	12
PS III	0.5	0.5	6.7	6.8	2.7	0.7	2.5	10

[a] This fraction only showed very low background color formation

fucose and X were present in equimolar amounts. This fraction also contained a firm amount of uronic acid, while the hexose content is relatively low.

PS III contained low molecular weight components, which were presumably split off during the hydrolysis in acetic acid. The difference in composition between XPS, PS I and PS II suggests that 3 different types of polysaccharide are produced by *Rhizobium leguminosarum.*

The presence of an antigenic polysaccharide in rhizobial cell walls of non-O-antigenic (LPS) nature was recently demonstrated by Humphrey and Vincent [18] who regarded it to be of microcapsular nature. They showed that it was able to prevent cross agglutination by masking the O-antigenic specificity of whole cells tested. Even exhaustive washing of cells in cold saline could not remove the component while it was absent in purified exopolysaccharide proving that it was not of exopolysaccharide origin.

The lectins isolated from pea seed precipitated undegraded LPS and PS I but not the lipopolysaccharide specific components containing PS II. XPS and all blanks were negative in this test. The interaction between lectins and *Rhizobium* as demonstrated by several investigators [5,6] was shown to be due to polysaccharide of capsular [7] or O-antigenic [8] origin.

The discrepancy between these two results can be explained by the presence of the PS I fraction in both preparations. In particular the LPS tends to form aggregates in aqueous solution and entraps easily other components which are hard to remove without degrading the LPS by splitting the hydrophylic polysaccharide part from lipid A. To what extent the lectins binding PS I only functions as a recognition factor or is actively involved in the infection process of the bacterium has still to be decided.

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