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Legume lectins interact with muramic acid and *N*-acetylmuramic acid

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The inhibitory potency of both muramic acid (MurAc) and *N*-acetylmuramic acid (MurNAc) on various legume lectins, including Glc/Man- and Gal/GalNAc-specific lectins, was investigated by a haemagglutination inhibition technique. Data indicated that many lectins, especially those specific for Glc/Man, specifically interact with MurAc and MurNAc often to a greater extent than with other monosaccharides and their derivatives, such as *N*-acetylglucosamine (GlcNAc) and sialic acid. Glc/Man-specific lectins were also shown to interact with the muramyl-dipeptide MurNAc-D-Ala-D-isoGln. These interactions could explain why various lectins readily agglutinate some bacterial strains of which cell walls contain peptidoglycans with high amounts of MurNAc.

Muramic acid; *N*-Acetylmuramic acid; Muramyl-dipeptide; Inhibition of haemagglutination; Legume lectin

1. INTRODUCTION

To date, interactions between legume lectins and simple or complex sugars have been fairly well documented, especially by checking the ability of different mono- or oligosaccharides to inhibit the haemagglutinating power of various lectins towards either human or animal erythrocytess [1]. According to their broad sugar specificities determined by this hapten-inhibition technique, lectins from the *Leguminosae* were classified into five different groups [1,2]. Most of the lectins identified so far, fall into two of these groups, corresponding respectively to the glucose/mannose-binding lectin (Glc/Man-specific lectins) and the galactose/*N*-acetylgalactosamine-binding lectins (Gal/GalNAc-specific lectins). However, to our knowledge, no systematic investigation was performed on the recognition by legume lectins of muramic acid (MurAc) and *N*-acetylmuramic acid (MurNAc). These two monosaccharides are widely distributed in the cell wall of bacteria [3], and their interaction with lectins could explain the previously reported agglutination of various bacteria by legume lectins [4,5].

2. MATERIALS AND METHODS

2.1. *Monosaccharides and muramyl-dipeptides*

All monosaccharides used in this study: D-glucose (Glc), D-mannose (Man), D-galactose (Gal), glucosamine (GlcN), galactosamine (GalN), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), *N*-acetylneuraminic acid (NeuAC), α-methyl-mannopyranoside (MeMan), muramic acid (MurAc) and *N*-acetylmuramic acid (MurNAc), were purchased from Sigma. Muramyl-dipeptides: MurNAc-L-Ala-D-isoGln (MDP L-D or adjuvant peptide), MurNAc-D-Ala-D-isoGln (MDP D-D) and MurNAc-L-Ala-L-iso-Gln (MDP L-L), were purchased from Sigma.

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2.2. *Isolation of lectins/isolectins*

The Glc/Man-specific lectins from *Lathyrus ochrus* (LoLI + LoLII), *L. tingitanus* (LtinL), *Pisum sativum* (PsA) and *Lens culinaris* (LcA), were isolated from seed meal extracts by affinity chromatography on Sephadex G100 (Pharmacia) and subsequent elution with 0.1 M glucose [6]. The separation of the two *L. ochrus* isolectins, LoLI and LoLII, was performed by chromatofocusing on PBE 94 (Pharmacia) in the pH range between 8.4 and 5.0, as described in [7]. The two Gal/GalNAc-specific lectins from *Butea frondosa* (BfL) and *Glycine max* (SBA), were purified from seed meal extracts by affinity chromatography on lactose immobilized on 4% crosslinked agarose (Pierce) [8] and on galactosamine derivatized CH-Sepharose 4B (Pharmacia) [9], respectively. Lectins from *Dolichos biflorus* (DbL) and *Erythrina corallodendron* (EcoL), were purchased from Sigma.

2.3. *Inhibition of haemagglutination*

The hapten-inhibition technique was carried out in U-bottomed micro-plates (Flow Laboratories). The wells were filled with 25 μl of 2-fold serial dilutions of 10 mM hapten in Tris-buffered saline (pH 7.2). Twenty five μl of lectin solution in TBS, corresponding to 4 haemagglutination doses, were added and after a 1 h incubation period at room temperature, 25 μl of a 1% suspension of human ORh+ erythrocytes (AlRh+ erythrocytes for DbL) in TBS were added. After mixing, inhibition of haemagglutination was estimated 2 h and 12 h later, respectively. Results were expressed as the minimum concentration (mM) of hapten required to completely inhibit 4 haemagglutination doses. Account was taken of the 3-fold dilution caused by the addition of lectin and erythrocytes.

3. RESULTS

The inhibitory potency of 11 different monosaccharides towards various Glc/Man-specific lectins (Table I), showed that both MurAc and MurNAc behave as potent inhibitors of all the assayed lectins, except for the

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Table I

Inhibition of Glc/Man- and Gal/GalNAc-specific legume lectins by monosaccharides

Lectins	Specificity	Inhibitory concentration (mM)										
		MeMan	Man	Glc	GlcN	GlcNAc	MurAc	MurNAc	Gal	GalN	GalNAc	NeuAc
LoLI	Glc/Man	0.21	1.65	3.3	>3.3	>3.3	0.41	3.3	>3.3	>3.3	>3.3	3.3
LoLII	Glc/Man	0.21	1.65	1.65	>3.3	>3.3	1.65	3.3	>3.3	>3.3	>3.3	3.3
LtinL	Glc/Man	0.41	1.65	>3.3	>3.3	>3.3	0.21	1.65	>3.3	>3.3	>3.3	1.65
PsA	Glc/Man	0.21	1.65	>3.3	>3.3	>3.3	0.41	0.82	>3.3	>3.3	>3.3	0.82
LcA	Glc/Man	0.82	3.3	>3.3	>3.3	>3.3	3.3	3.3	>3.3	>3.3	>3.3	0.82
DbL	Gal/GalNAc	>3.3	>3.3	>3.3	>3.3	>3.3	1.65	1.65	0.41	1.65	0.21	0.21
EcoL	Gal/GalNAc	>3.3	>3.3	>3.3	>3.3	>3.3	>3.3	>3.3	1.65	3.3	0.41	3.3
SBA	Gal/GalNAc	>3.3	>3.3	>3.3	>3.3	>3.3	1.65	1.65	0.82	0.82	0.05	3.3
BfL	Gal/GalNAc	>3.3	>3.3	>3.3	>3.3	>3.3	1.65	0.82	1.65	1.65	0.21	1.65

lentil lectin (LcA). MurAc was found to be 2- to 8-fold less inhibitory than methyl α-mannoside, one of the most potent inhibitors of these Glc/Man-specific lectins, but 4- to 8-fold more inhibitory than glucose. However MurAc was a somewhat better inhibitor than methyl α-mannoside towards the *Lathyrus tingitanus* lectin (LtinL). MurAc was 2- to 8-fold more inhibitory than MurNAc. Sialic acid had approximately the same activity as MurNAc. Lentil lectin (LcA), exhibited a different behaviour, since it was equally well weakly inhibited by MurAc and MurNAc. From these data, the inhibitory potencies of the checked monosaccharides are: MeMan>MurAc>Man,NeuAc>MurNAc>Glc.

No steric hindrance introduced by the presence of the CH3-C*H-COOH group at the C-3 position of MurAc, is likely to hamper the binding to the monosaccharide-binding site. This substitution introduces an apolar CH3 group within a region where the binding of glucose, mannose and their methyl-derivatives was shown to involve mainly hydrophilic amino acid residues, e.g. Arg^{228} in Con A [10] or Gly^{99} in LoLI [11]. By comparison with MurAc, the 2- to 8-fold decrease of the inhibitory potency of MurNAc suggests that the acetylated group at the C-2 position could create a steric hindrance responsible for the lower binding of MurNAc, although the C-2 substituents of both glucose and mannose did not interact with the monosaccharide-binding site of Con A [10] and LoLI [11]. LcA, which interacts equally well with MurAc and MurNAc, has a different behaviour. Three (DbL, SBA, Bfl) of the four checked Gal/GalNAc-binding lectins, also reacted with MurAc and MurNAc, but at a lesser extent (Table I). Accordingly, *N*-acetyl-galactosamine, their strongest inhibitor, was found to be 8- to 64-fold more inhibitory, while galactose was only 2-fold more inhibitory. However, no difference occurred between MurAc, MurNAc and sialic acid, which were found to be equally well inhibitory. Lectin from *E. corallodendron* was strongly inhibited by *N*-acetylgalactosamine and, to a lesser extent, by galactose, but was almost unreactive towards MurAc and MurNAc (Table I). The inhibitory potencies of the monosaccharides range as follows: GalNAc>Gal>GalN,NeuAc>MurNAc>MurAc. Out of the three myramyl-dipeptides, only the diastereo-isomeric MurNAc-D-Ala-D-isoGln (MDP D-D), inhibited the Glc/Man-specific lectins but was almost unreactive towards other Gal/GalNAC-specific lectins (Table II). The two other muramyl-dipeptides, MurNAc-L-Ala-D-isoGln (MDP L-D) and MurNAc-L-Ala-L-isoGln (MDP L-L), were weakly active towards all the checked lectins. These findings suggest that some stereospecificity might occur in the recognition of the MurNAc-bearing peptides by legume lectins.

4. DISCUSSION

Until now, very few lectins were shown to interact with MurAc- or MurNAc-containing saccharides or glycopeptides. GlcNAc-specific lectins from thorn apple (*Datura stramonium*) and potato (*Solanum tuberosum*), both from the family *Solanaceae*, were reported to interact with MurNAc-containing bacterial cell-wall oligosaccharides [12,13]. Wheat germ agglutinin (WGA) precipitated bacterial peptidoglycan and teichoic acid [14], and reacted with the bacterial cell-wall tetrasaccharide

Table II

Inhibition of Glc/Man- and Gal/GalNAc-specific legume lectins by muramyl-dipeptides

Lectin	Specificity	Inhibitory concentration (mM)		
		MDP D-D	MDP L-D	MDP L-L
LoLI	Glc/Man	0.21	3.3	>3.3
LoLII	Glc/Man	0.21	1.65	>3.3
LtinL	Glc/Man	0.21	3.3	>3.3
PsA	Glc/Man	0.21	>3.3	>3.3
LcA	Glc/Man	1.65	>3.3	>3.3
DbL	Gal/GalNAc	3.3	>3.3	>3.3
EcoL	Gal/GalNAc	>3.3	>3.3	3.3
SBA	Gal/GalNAc	3.3	3.3	>3.3
BfL	Gal/GalNAc	3.3	3.3	3.3

GlcNAc (β1-4)MurNAc (β1-4)GlcNAc(β1-4)MurNAc [15]. Limulin was also reported to interact with teichoic acid [16,17]. Other invertebrate lectins from crabs [18,19] and scorpions [20,21], similarly interacted with MurAc and MurNAc. Owing to their ability to recognize MurAc and, at a lesser extent, MurNAc and GlcNAc, which are components of peptidoglycans of the cell wall of bacteria and especially of the Gram$^+$ bacteria, legume lectins are susceptible to interact with various microorganisms. This interaction could be of importance in relation to the behaviour of microorganisms present in soils towards their plant partners, e.g. to promote their saprophytic, symbiotic or pathogenic propensities.

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