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# Carbohydrate binding properties of banana (*Musa acuminata*) lectin

## II. Binding of laminaribiose oligosaccharides and β-glucans containing β1,6-glucosyl end groups

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This paper extends our knowledge of the rather bizarre carbohydrate binding poperties of the banana lectin (*Musa acuminata*). Although a glucose/mannose binding protein which recognizes α-linked *gluco*-and *manno*-pyranosyl groups of polysaccharide chain ends, the banana lectin was shown to bind to internal 3-*O*-α-D-glucopyranosyl units. Now we report that this lectin also binds to the reducing glucosyl groups of β-1,3-linked glucosyl oligosaccharides (e.g. laminaribiose oligomers). Additionally, banana lectin also recognizes β1,6-linked glucosyl end groups (gentiobiosyl groups) as occur in many fungal β1,3/1,6-linked polysaccharides. This behavior clearly distinguishes the banana lectin from other mannose/glucose binding lectins, such as concanavalin A and the pea, lentil and *Calystegia sepium* lectins.

*Keywords*: *Musa*; banana; lectin; β1, 6-linked glucosyl; laminaribiose.

In the companion paper we provided data establishing that the mannose/glucose binding banana lectin is capable of recognizing internal α1,3-linked glucosyl residues as they occur in certain fungal polysaccharides such as elsinan and nigeran [1]. First isolated by Koshte and colleagues [2], the banana lectin is a tetrameric protein, composed of four identical subunits with $M_r$ of 15 kDa. It has been cloned and shown to have amino acid sequence similarity to lectins of the jacalin-related group [3].

The banana lectin appears to be quite 'promiscuous' in its activity in that, although it is a mannose/glucose-binding lectin, and shares many properties with mannose/glucose recognizing legume lectins (e.g. conA), it also displays some very novel binding behavior, as we will report in this paper; namely, its interaction with reducing 3-O-linked β-D-glucosyl residues, and nonreducing 6-*O*-β-D-glucosyl end groups.

## MATERIALS AND METHODS

### Carbohydrates

Most mono- oligo and polysaccharides and their derivatives were available from previous studies. Laminari-biose, -triose, and -heptaose were purchased from Dextra Laboratories, Ltd (Reading, UK). Laminaran and its oligosaccharides were reduced with $NaBH_4$, and nonreducing branched stubs were removed from laminaran by periodate oxidation, $NaBH_4$ reduction, and mild acid hydrolysis [4]. The β-glucans from barley, the mushroom *Pleurotus ostreatus* and *Alcaligenes faecalis* (curdlan) were purchased from Sigma. Samples of *Phytophthora megasperma* f. sp. *glycinea* oligosaccharides were the gift of M. Hahn of the Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA.

### Lectins

Banana lectin was prepared as described [3]. ConA, the pea, lentil, and *Calystegia sepium* lectins were availalble from previous studies [1,3].

### Quantitaive precipitation assays

These were performed by a microprecipitation procedure essentially as described by So and Goldstein [5] with some modifications [1].

### Isothermal titration calorimetry

Titrations were performed in a CSC 4200 microcalorimeter (Calorimetric Sciences, Inc., Spanish Fork, UT) as described [1].

### Affinity column chromatography

A slurry of finely powdered, water-insoluble polysaccharides (200–300 mg) in NaCl/$P_i$ was packed into a small glass column over a 1-cm layer of cellulose. Lectins (200–500 μg) in NaCl/$P_i$ (1 mL) were added to the equilibrated column and eluted at a flow rate of 1 mL·min$^{-1}$. monitoring protein at $A_{280}$. When the

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*Abbreviations*: conA, concanavalin A.

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**Table 1. Binding constants of carbohydrates to banana lectin.** NR, no measurable binding.

| Saccharide | $K_d$ (mM) | $K_a$ ($10^2$ M$^{-1}$) |
|---|---|---|
| Me-α-Glc*p* | 7.5 | 1.33 |
| Me-β-Glc*p* | 24.3 | 0.41 |
| 3-*O*MeGlc | 5.41 | 1.85 |
| Laminaribiose (Glcβ1,3Glc) | 1.02 | 9.73 |
| Laminaritriose | 1.18 | 8.5 |
| Laminariheptaose | 1.05 | 9.53 |
| Laminaribiitol | NR | – |
| Laminariheptaitol | NR | – |
| Gentiobiose (Glcβ1,6Glc) | 22.2 | 0.45 |

absorbance reached the baseline (< 0.05) methyl α-mannoside was added to displace any bound protein.

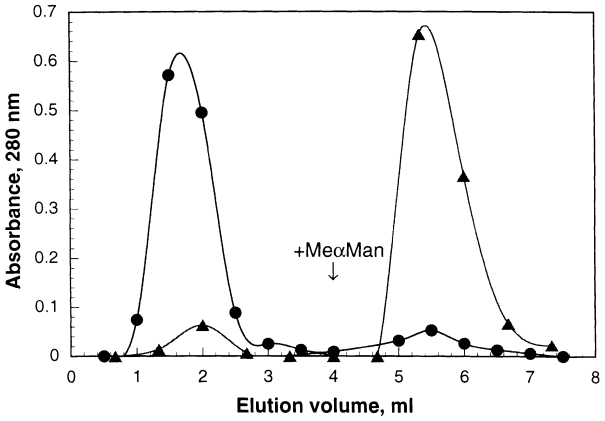


**Fig. 2. Binding and elution of banana lectin and conA to insoluble β-glucan from *Pleurotus ostreatus*.** Each protein (200–500 μg) was added to the column (0.6 × 5 cm) and monitored by absorbance at 280 nM. Methyl α-mannoside, 0.1 M (arrow) was added when the absorbance reached a baseline value. ●, conA;▲, banana lectin.

## RESULTS

In the companion comunication [1] we reported that laminaribiose (Glcβ1,3Glc) was a relatively good carbohydrate ligand for the banana lectin with a binding constant of $8.3 \times 10^2$ M$^{-1}$. In this paper we amplify and extend the significance of this finding. Table 1 and Fig. 1 provide a list of several laminaribiose oligomers together with their structures and binding constants determined by isothermal titration calorimetry. It will be noted that laminari-biose, -triose and -heptaose all have approximately the same binding constant. On the other hand, the corresponding alditol reduction products of the biose and heptaose did not bind to the banana lectin. These results suggest that it is the reducing glucose unit of these oligosaccharides with which the banana lectin interacts. This is consistent with the finding that the banana lectin binds 3-*O*-methyl glucose and recognizes internal 3-*O*-linked α-D-glucosyl/mannosyl residues [1].

To ascertain whether internal β1,3-linked glucosyl residues are recognized by the banana lectin, we examined its interaction with several polysaccharides containing these glucosidic linkages. Some of these polymers were insoluble and were assayed for their ability to bind the banana lectin by packing them into a small glass column and percolating the lectin through the finely divided polysaccharide matrix, monitoring the effluent for protein by absorbance at 280 nm. Methyl α-mannoside (0.1 M) was added to the column to displace any bound lectin. In this fashion it was

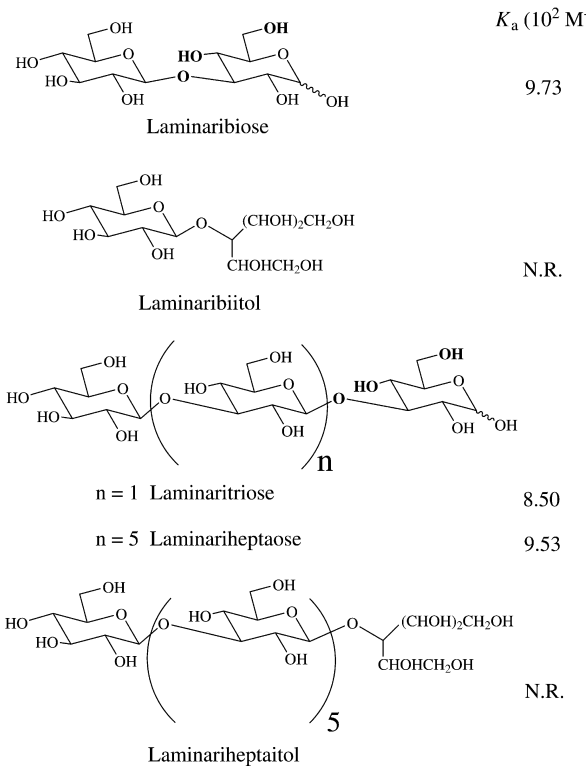


**Fig. 1. Structures of laminaran oligosaccharides and their binding constants determined calorimetrically.** The hydroxyl groups and glucosidic O-atom believed to be involved in binding to the banana lectin are printed in bold type.

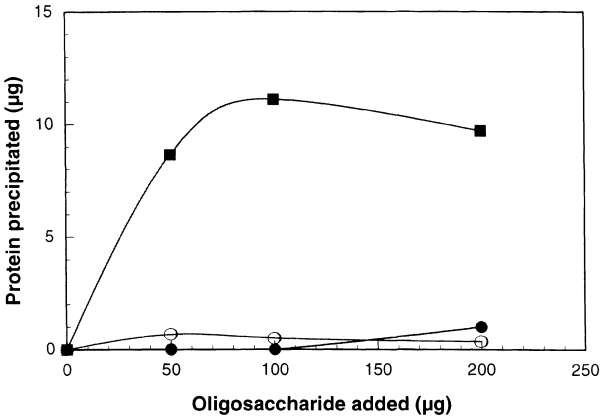


**Fig. 3. Quantitative precipitation reaction of banana lectin with oligosaccharides from *Phytophthora megasperma* β-glucan.** Each tube contained 20 μg of lectin, and varying amount of oligosaccharides of the indicated degree of polymerization (DP): ●, DP 7; ○, DP 8–16; ■, DP > 20.

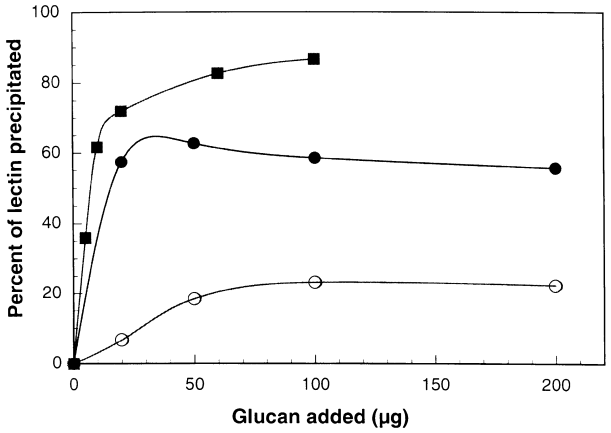


**Fig. 4. Quantitation precipitation reaction of banana lectin with laminaran and schizophyllan.** Each tube contained 15 μg banana lectin for reaction with laminaran, and 26 μg for reaction with schizophyllan. ●, schizophyllan; ■, laminaran; ○, laminaran (debranched)

demonstated that curdlan, a linear β1,3-glucan, and cellulose (β1,4-glucan) failed to bind the banana or plantain lectins. Similarly, barley glucan, a soluble, linear β-glucan containing β1,3- and β1,4-glucosyl residues, failed to generate a precipitin curve with the lectin.

On the other hand, the *Pleurotus ostreatus* mushroom polysaccharide, an insoluble β1,3-glucan containing β1,6-glucosyl stubs, bound the banana lectin and was eluted by methyl α-mannoside (Fig. 2). Additionally, we assayed two mixtures of soluble branched oligosaccharides obtained from the *Phytophthora megasperma* fungus by acid hydrolysis; they contains both β1,6 and β1,3 glucosidic bonds. The fraction with an average degree of polymerization of >20mers gave the precipitation curve shown in Fig. 3, whereas the fraction with oligosaccharides of chain length 8–16 did not form a precipitate with the lectin. Interestingly, the glucoheptaose and octaose, which stimulate the formation of phytoalexins in soybeans, did not precipitate the banana lectin. This is because these oligosaccharides, which consist of a linear chain of five glucosyl residues linked β1,6 with two glucosyl branches linked β1,3, possess only a single β1,6-end group.

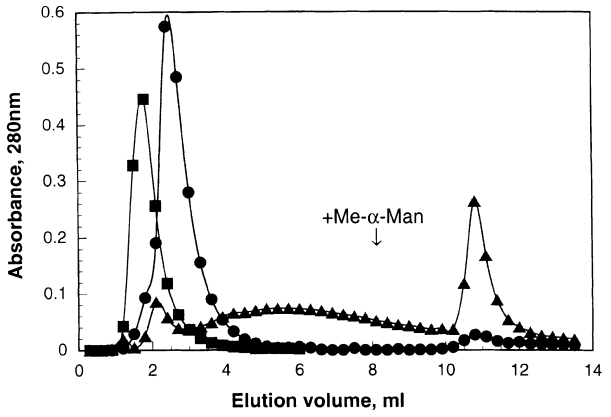


**Fig. 5. Binding of banana lectin and conA to gentiobiose–Sepharose.** Experiment performed as in Fig. 2. ●, conA; ▲, banana lectin; ■, bovine serum albumin.

Soluble laminaran, a linear β1,3-glucan polymer bearing several β1,6-linked glucosyl end groups [7] generated a precipitin curve with the banana lectin, as did the fungal β-glucan schizophyllan (Fig. 4). The latter is a linear 1,3-linked β-glucan containing single β1,6-linked glucosyl stubs. Treatment of schizophyllan with periodate/borohydride (but without mild acid hydrolysis) converted the polysaccharide to the corresponding insoluble polyalcohol which, when packed into a column, bound less than 10% of the banana lectin. Furthermore, removal of most of the β1,6-glucosyl end groups from laminaran [4] greatly decreased its ability to precipitate the lectin (Fig. 4). On the other hand, conA, the lentil, pea and *Calystegia sepium* lectins, all mannose/glucose-binding lectins which share many carbohydrate binding properties with the banana lectin, did not bind to the *P. ostreatus* mushroom polysaccharide, or generate precipitin curves with intact laminaran or schizophyllan (data not shown). The difference in binding affinity between the banana lectin and conA is clearly depicted in Fig. 5. When solutions of these lectins were passed through a column of gentiobiosyl (Glcβ1,6Glc) Sepharose, conA was slightly retarded, whereas most of the banana lectin bound to the gentiobiosyl matrix and was displaced by methyl α-mannoside. This indicates that the banana lectin is capable of forming a three dimensional network by crosslinking multiple β1,6 glucosyl end groups in polysaccharides containing these carbohydrate groups.

## DISCUSSION

The experiments described in this paper establish two fundamental features of the banana lectin: (a) It is capable of binding to the reducing sugar residues of oligosaccharides which are glycosidically β-linked at the C-3 OH group of glucose, and presumably, mannose molecules; and (b) It can recognize, interact and precipitate with complex carbohydrates containing multiple β1,6-linked glucosyl (gentiobiosyl) end groups.

Reduction by $NaBH_4$ converts the reducing ends of the β1,3-linked oligosaccharides (laminaridextrins) to alditol groups, thus abolishing their reaction with the banana lectin and confirming that it is the 3-O-linked reducing glucosyl groups with which the lectin interacts.

The binding of lectins to high molecular weight, insoluble β1,3-glucans was assayed by binding of the lectins to the insoluble matrix and elution of any bound lectin with methyl α-mannoside. The banana lectin did not bind to curdlan, a totally linear β1,3-glucan; the lectin eluted with the void volume of the column. On the other hand, those β1,3-glucans containing β1,6 glucosyl end groups bound or precipitated the banana lectin, and that activity was lost upon removal of the β1,6 end groups by periodate oxidation and subsequent reduction and/or cleavage. It should further be noted that soluble laminaran predominantly contains a mannitol residue at the reducing terminus [6], so that binding by a 3-substituted reducing glucose moiety is not likely to be involved in the precipitation reaction. Displacement of the banana lectin from a column of the insoluble *P. ostreatus*

mushroom polysaccharide by methyl α-mannoside strongly suggests that the carbohydrate binding sites of the banana lectin recognize both α-linked glucosyl/mannosyl end groups (e.g. glycogen and yeast mannan, respectively) as well as multiple 6-O-β-linked glucosyl residues which occur as end groups in polysaccharides. This behavior also significantly distinguishes the banana lectin from conA.

To the best of our knowledge, this is the first demonstration of a plant lectin shown to bind to the reducing moiety of 3-O-β-linked glucosyl residues, and to precipitate or bind to multiple 6-O-β-linked glucosyl end groups. However, there have been several reports of receptors (lectins) for 1,3 β-glucans in both vertebrate and invertebrate blood cells, e.g. from crayfish and several insects [7,8]. These reports are based on affinity isolation of the lectins with laminaran, and do not consider the possible involvement of β1,6-glucosyl end groups.

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