Carbohydrate binding properties of banana (*Musa acuminata*) lectin
I. Novel recognition of internal α1,3-linked glucosyl residues

Hanqing Mo[1], Harry C. Winter[1], Els J. M. Van Damme[2], Willy J. Peumans[2], Akira Misaki[3] and Irwin J. Goldstein[1]

[1]*Department of Biological Chemistry, The University of Michigan Medical School, USA;* [2]*Laboratorium voor Fytopathologie en Plantenbescherming, Katholieke Universiteit Leuven, Belgium;* [3]*Osaka City University, Sumiyoshi, Japan*

Examination of lectins of banana (*Musa acuminata*) and the closely related plantain (*Musa* spp.) by the techniques of quantitative precipitation, hapten inhibition of precipitation, and isothermal titration calorimetry showed that they are mannose/glucose binding proteins with a preference for the α-anomeric form of these sugars. Both generate precipitin curves with branched chain α-mannans (yeast mannans) and α-glucans (glycogens, dextrans, and starches), but not with linear α-glucans containing only α1,4- and α1,6-glucosidic bonds (isolichenan and pullulan). The novel observation was made that banana and plantain lectins recognize internal α1,3-linked glucosyl residues, which occur in the linear polysaccharides elsinan and nigeran. Concanavalin A and lectins from pea and lentil, also mannose/glucose binding lectins, did not precipitate with any of these linear α-glucans. This is, the authors believe, the first report of the recogniton of internal α1,3–glucosidic bonds by a plant lectin. It is possible that these lectins are present in the pulp of their respective fruit, complexed with starch.

Keywords: *Musa*; banana; lectin; carbohydrate-binding; α1, 3-linked glucosyl.

Plant lectins provide a rich source of carbohydrate-recognizing protein reagents for the glycobiologist. Lectins with specificity toward a large number of different mono-saccharides, oligosaccharides and polysaccharides have been described, but until now, no plant lectin that recognizes and binds to internal α1,3-linked glucosyl residues has been reported.

The banana lectin (*Musa acuminata*), first isolated by Koshte and colleagues [1], is a homotetramer, subunit M_r 15 kDa. It was reported to induce the formation of IgG_4 antibodies, to be a T-cell mitogen, and to bind mannose and some of its oligosaccharides [1,2]. Peumans *et al.* [3] have cloned the gene that encodes the banana lectin and found it to belong to a family of jacalin-related lectins. We have conducted an in-depth investigation of the carbohydrate binding properties of the banana lectin and the related lectin from the plantain (*Musa* spp.) with some novel and unexpected findings. This communication reports our finding that a plant lectin interacts with internal α1,3-linked glucosyl residues, which occur in several poly-saccharides and glycoproteins.

Correspondence to I. J. Goldstein, Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109-0606, USA. Fax: + 734 7634581, Tel.: + 734 7633511, E–mail: igoldste@umich.edu

MATERIALS AND METHODS

Carbohydrates

Most monosaccharides, oligosacchaarides and polysacchar-ides, and their derivatives were available from previous studies.

Lectins

Banana and plantain lectins were prepared as described [3]. Lentil and pea lectins, and concanavalin A (conA) were purchased from EY Laboratories, Inc. (San Mateo, CA, USA). The *Calystegia sepium* lectin was availalble from a previous study [4].

Quantitative precipitation and hapten inhibition assays

These were performed by a microprecipitation procedure essentially as described by So and Goldstein [5] with some modifications. Briefly, varying amounts of polysaccharides, ranging from 0 to 100 μg were added to 15–20 μg of purified lectin (banana, lentil, pea, *C. sepium* or conA) in a total volume of 120 μL of NaCl/P_i (10 mM phosphate-buffered saline containing 0.1 mM $CaCl_2$ and 0.04% NaN_3, pH 7.2). After incubation at 37° for 1 h, the reaction mix-tures were stored at 4° for 48 h. The precipitates formed were washed three times with 150 μL of ice-cold NaCl/P_i, dissolved in 0.05 M NaOH and assayed for protein by Lowry's method [6] using bovine serum albumin as standard.

For hapten inhibition assays, increasing amounts of various haptenic saccharides were added to the reaction mixtures consisting of banana lectin (15 μg) and yeast mannan (20 μg) in a final volume of 120 μL of NaCl/P_i, pH 7.2. After processing as above for precipitation assays, and determining the protein content of the precipitates,

inhibition curves were constructed from which the concentration of each haptenic sugar causing 50% inhibition of precipitation (I_{50}) was estimated.

Affinity column chromatography

A slurry of the finely pulverized water-insoluble polysaccharides (200–300 mg) was packed into a small glass column (0.8 × 4 cm) and equilibrated with NaCl/P_i. Lectins (300–500 µg) in NaCl/P_i were added to the column followed by elution with NaCl/P_i. When A_{280} reached the baseline (< 0.050), methyl α-mannoside (200 mM) was added and the displaced proteins were monitored similarly.

Periodate oxidation/borohydride reduction of polysaccharides

Periodate oxidation/borohydride reduction of nigeran and elsinan were carried out by the procedure of Goldstein *et al.* [7]. Partial hydrolysis of a small portion of the polysaccharide (20 mg) was carried out in 0.5 M HCl for 8 h. at room temperature (25°). This was followed by deionization and thin layer chromatography in the solvent system water/acetone/*n*-butanol (2 : 3 : 5, v/v/v) with glucosyl erythritol and erythritol as standards.

Isothermal titration calorimetry

Titrations were performed in a CSC 4200 microcalorimeter (Calorimetric Sciences, Inc., Spanish Fork, UT, USA) at 25.00° using a 1.3-mL cell in the constant-volume (overfill) mode. Banana lectin in NaCl/P_i at a subunit concentration of 0.12–0.5 mM was titrated with ligands in the same stock of NaCl/P_i at concentration of 10–100 mM, depending on the expected K_a value. Titrations were generally carried out with 25 additions of 5 µL, each at 300 s intervals. Integration of the raw titration data and curve fitting were performed using the BINDWORKS software supplied with the instrument. Heat values were corrected for the heat of dilution of the titrant determined in a separate blank titration, or estimated by extrapolation of the titration curve. In some instances, K_a and ΔH_{max} values were calculated from a Lineweaver-Burk plot of $1/\Sigma\Delta H$ vs. reciprocal of the total ligand concentration at each titration point.

RESULTS

The banana lectin (*Musa acuminata*) generated quantitative precipitation curves with the highly branched α-mannan from yeast (*Saccharomyces cerevisiae*), rabbit liver glycogen, banana starch, floridean starch from red algae, a sample of amylopectin β-limit dextrin, and several dextrans (all branched α-D-glucans with numerous α-D-glucosyl end groups) (Fig. 1A,B). Dextran B-1355-S is very highly branched [8] whereas the dextrans from *Streptococcus bovis* [9] and *Streptococcus* sp. [10]. contain only a few branch points. This is reflected in the different shapes of the precipitin curves: dextran B-1355-S gave a steeper ascending curve and precipitated a greater amount of protein than the other two dextrans (Fig. 1A). The banana lectin did not give a precipitin curve with two linear α-D-glucans, isolichenan, which consists of maltotriose groups separated by α1,6-linkages and pullulan, which contains maltosyl

residues also separated by α1,6-glucosidic bonds. Of a series of plant and bacterial levans, both linear and branched β2,6-D-fructofuranosyl-containing polysaccharides, only those from *Aerobacter levanicum* and *Zymonas mobilis* reacted to give precipitin curves (not shown). A galactomannan from *Cassia alata,* which contains multiple stubs of α-D-galactosyl-groups, failed to react with the banana lectin. Precipitin tests with the related plantain (*Musa* sp.) lectin showed a qualitatively similar pattern of precipitation (data not shown).

Two precipitin curves of interest (Fig. 1B) were the ones generated by the bovine serum albumin conjugate containing multiple units of the disaccharide Glcα1,2Gal, the glycosyl groups found in collagen [11]; and by *Pneumococcus* Type XII polysaccharide, which contains kojibiosyl residues (Glcα1,2Glc) within its linear structure [12,13]. This disaccharide unit allows reaction with the banana lectin at its α1,2-linked glucosyl residues via its three available hydroxyl groups at C3, 4 and 6.

Unexpectedly, both conA and the banana lectin precipitated with elsinan (Fig. 2), an essentially linear polysaccharide reported to consist of maltotriose and maltotetraose units in a ratio of 2 : 1 joined by α1,3-linkages [14]. As neither of these lectins bind to internal maltosyl residues, we considered that the lectins might be reacting with the α-1,3-glucosyl groups. This was a reasonable speculation for the banana lectin because it reacted well with 3-*O*-methyl glucose (see below). However, conA does not bind 3-*O*-methyl glucose [15], suggesting that elsinan might contain a few branch points, or a branched component with which conA was reacting. To test this hypothesis, we passed
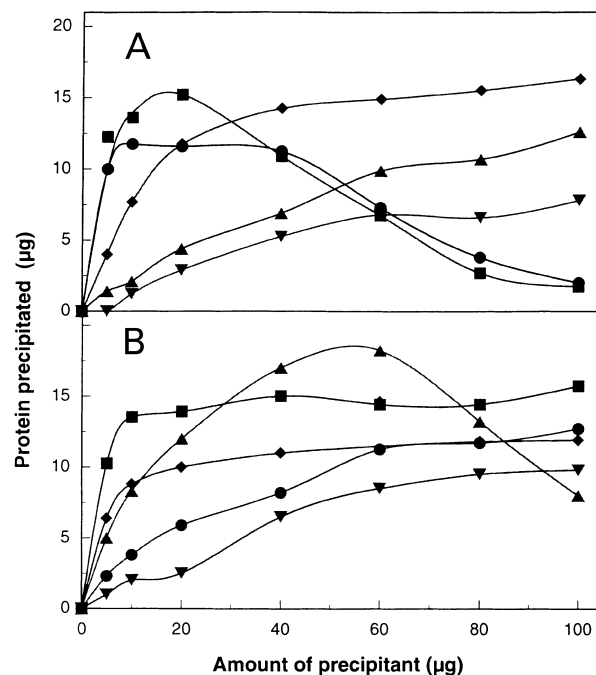

Fig. 1. Quantitative precipitation curves of banana lectin with various polysaccharides. (A) ■, dextran B 1355-S; ●, yeast mannan; ◆, rabbit glycogen; ▲, *Streptococcus* sp. dextran; ▼, *S. bovis* dextran. (B) ■, β-limit dextrin; ▲, Glc α1,2Gal β-BSA conjugate; ◆, pneumococcal type 12 polysaccharide; ●, floridean starch; ▼, banana starch.

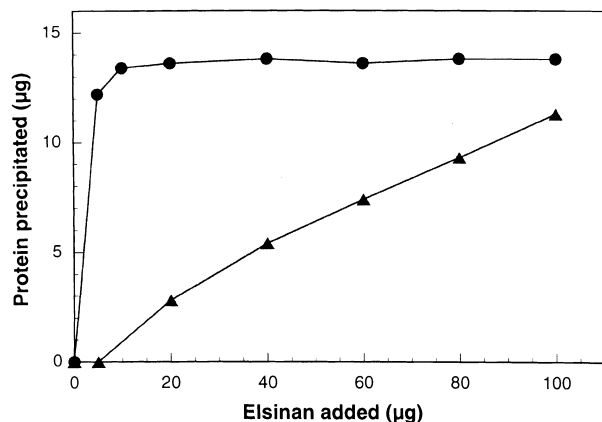Fig. 2. **Quantitative precipitation curves of banana lectin or con. A with elsinan.** ●, banana lectin; ▲, conA.

Fig. 4. **Affinity chromatography of banana lectin on insoluble, linear α1,3-glucan from *Stretococcus salvarius*.**

a solution of elsinan through a column of conA-Sepharose, collecting the effluent and then eluting any bound material with buffer containing 100 mM methyl α-D-mannopyranoside. The two fractions from the separation were pooled separately, dialyzed and lyophylized. Approximately 2% of the elsinan preparation was retained on the conA-Sepharose column and displaced by methyl α-mannoside. The fraction that passed through the conA-Sepharose column did not react with conA, but retained its reactivity with the banana lectin (not shown), whereas both lectins generated precipitin curves with the fraction displaced by methyl α-mannoside (Fig. 3). The conclusion that elsinan preparations contain a small amount of a branched component was confirmed by methylation analysis of the conA-bound fraction, which indicated a branched α-glucan containing α1,3 and 1,4 glucosidic bonds with branch points at the O-6 position of the α1,3-linked glucosyl residues.

Neither the lentil nor the pea lectins generated precipitation curves with elsinan. Both of these mannose/glucose-binding lectins are inhibited by 3-*O*-methyl glucose [16]. The lectin from *C. sepium* likewise failed to precipitate
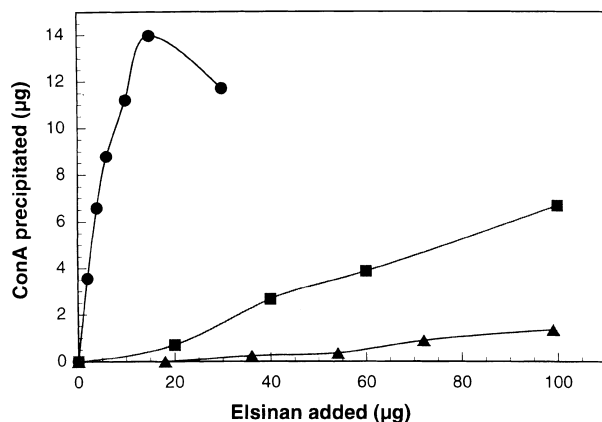

Fig. 3. **Quantitative precipitation curves of conA by elsinan fractionated on conA-Sepharose.** ●, elsinan bound to and eluted from conA-Sepharose by methyl α-mannoside; ■, native unfractionated elsinan; ▲, elsinan preparation that passed through a column of conA-Sepharose.
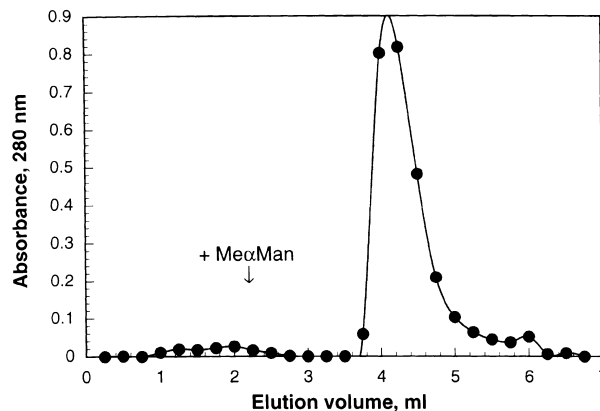
with elsinan, although it belongs to the same family of jacalin-related lectins as does banana lectin [3].

A further indication that the banana lectin recognizes internal α1,3-glucosyl residues is afforded by its interaction with an α1,3 glucan from *Streptococcus salivarius*. This polysaccharide, as isolated, has a few α1,6-linked glucosyl branches. These were removed by Smith degradation [7]. The resulting insoluble product was subjected to methylation analysis giving only 2,3,4,6-tetra-*O*-methyl glucose and 2,4,6-tri-*O*-methyl glucose, confirming the linear nature of the 1,3-linked polymer. On affinity column chromatogaphy (Fig. 4) it is apparent that the lectin bound to the α1,3-glucan and was eluted with methyl α-mannoside.

Nigeran, the linear, water-insoluble α-glucan elaborated by *Aspergillus niger,* contains approximately equal proportions of α1,3- and α1,4-linkages which alternate. Both conA and the banana lectin bound to the finely powdered polysaccharide and were displaced by 200 mM methyl α-mannoside. It was expected that the banana lectin, but not conA, would bind to nigeran. We believe the conA binds to the non reducing maltosyl and/or nigerosyl end groups of nigeran, but not to the internal α1,3-linked glucosyl units. Nigeran was subjected to periodate oxidation/borohydride reduction and isolated as a soluble polyalcohol. Smith degradation (limited mild acid hydrolysis at room temperature), followed by thin layer chromatography gave a single component, 2-*O*-α-D-glucopyranosyl-D-erythritol with a trace of erythritol, confirming the alternating α1,3/1,4 glucosyl structure of the nigeran preparation. Banana lectin gave an extremely strong precipitation reaction with the polyalcohol whereas conA was essentially unreactive (Fig. 5).

Interestingly, the branched trisaccharide Manα1,6 (Manα1,3)Man generated a precipitation curve with the banana lectin, as did the branched pentamannose oligosaccharide (Fig. 6).

Sugar hapten inhibition of precipitation and isothermal calorimetric titration further confirmed the designation of the *Musa acuminata* lectin as a mannose/glucose binding lectin (Tables 1 and 2). Of the monosaccharide hexoses tested, only D-mannose and D-glucose inhibited the lectin-yeast mannan precipitation system. D-allose and D-galactose (the C-3 and C-4 epimers, respectively, of D-glucose), and D-talose (the C-4 epimer of D-mannose) were non
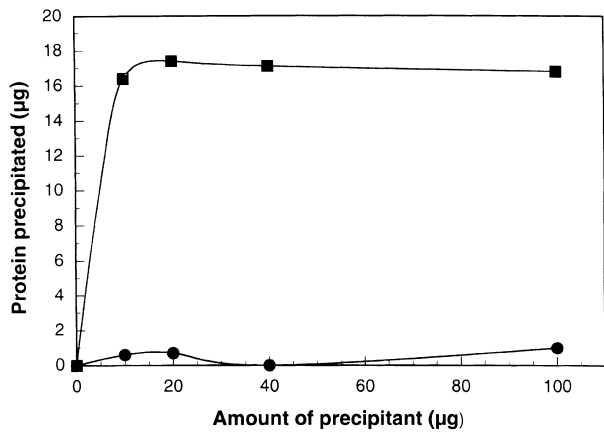
Fig. 5. Quantitative precipitation curve of banana lectin and conA by periodate-oxidized/borohyride-reduced nigeran. ■, banana lectin; ●, conA.
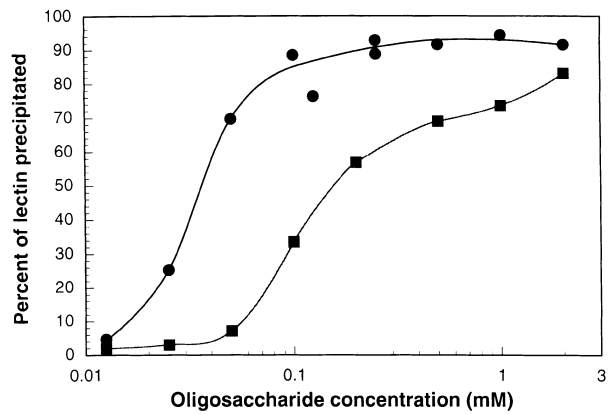

Fig. 6. Quantitative precipitation curves of two branched mannose oligosccharides with banana lectin. ●, branched pentasacchaide; ■, branched trisaccharide.

Table 1 Inhibition of precipitation of banana lectin with yeast mannan by mono- and oligosaccharides. ND, no inhibition detected at 100 mM. The following sugars also were non inhibitory at 100 mM: D-allose, D-arabinose, $N'N'$-diacetylchitobiose, methyl α-D-galactoside, lactose, melibiose, sucrose, D-talose, D-xylose and methyl α- and β-D-xylopyranosides.

Carbohydrate	I_{50} (mM)	Relative potency
Mannose	16.5	1.0
Me α-Man	7.8	2.1
Me β-Man	43.0	0.4
p nitrophenyl β-Man	12.8	1.3
1-deoxyMan (1,5-anhydro-D-mannitol)	16.0	1.0
2,5-anhydro-D-mannitol	ND	
Glucose	35.0	0.47
Me α-Glc	22.0	0.75
Me β-Glc	23% at 100 mM	
GlcNAc	33.0	0.5
1-deoxyGlc (1,5-anhydro-D-glucitol)	33.0	0.5
2-deoxyGlc	31.0	0.53
3-deoxyGlc	ND	
4-deoxyGlc	5% at 100 mM	
Me 6-deoxy α-Glc	5% at 100 mM	
2-O-Me Glc	38.0	0.43
3-O-Me Glc	40.0	0.40
4-O-Me Glc	5% at 100 mM	
6-O-Me Glc	ND	
3-deoxy-3-FGlc	59.0	0.28
6-deoxy-6-FGlc	ND	
Fructose	11.5	1.4
Me β-Frup	86% at 5 mM	> 3.3
Me α-Fru*f*	5% at 100 mM	
Man α1,2Man	8.5	1.9
Man α1,3Man	7.8	2.1
Man α1,6Man	8.2	2.0
Glc α1,2Glc (kojibiose)	16.0	1.0
Glc α1,3Glc (nigerose)	25.0	0.67
Glc α1,4Glc (maltose)	40.0	0.4
Glc α1,4Glc α1,4Glc(maltotriose)	34.0	0.49
Glc α1,6Glc (isomaltose)	18.7	0.9
Glc α1,6Glc α1,4Glc (panose)	24.0	0.69
Glc α,αGlc (α,α′-trehalose)	18.5	0.9
GlcNAc α1,6Gal	46.0	0.36
Glc β1,2Glc (sophorose)	54.0	0.3
Glc β1,3Glc (laminaribiose)	12.7	1.3
Glc β1,4Glc (cellobiose)	ND	
Glc β1,6Glc (gentiobiose)	40% at 100 mM	

Table 2 Binding constants of carbohydrates to banana and plantain lectins.

Saccharide	K_d (mM)	K_a (10^2 M^{-1})
Me-α-Man	3.0	3.33
Me-α-Man[a]	2.3	4.36
Manα1,3Man	2.7	3.72
Manα1,6Man	3.6	2.75
Galα1,3ManαOMe	2.7	3.65
Glucose	8.2	1.22
Me-α-Glc	7.5	1.33
Me-α-Glc[a]	6.0	1.68
Me-β-Glc	24.3	0.41
Me-β-Fruc*p*	1.96	5.10
Me-β-Fruc*p*[a]	1.77	5.64
3-OMeGlc	5.41	1.85
3-OMeGlc[a]	8.40	1.19
Kojibiose (Glcα1,2Glc)	1.84	5.43
Nigerose (Glcα1,3Glc)	5.49	1.82
Isomaltose (Glcα1,6Glc)	5.0	2.00
Isomaltotriose	7.63	1.31
Sophorose (Glcβ1,2Glc)	8.77	1.14
Laminaribiose (Glcβ1,3Glc)	1.20	8.3
Gentiobiose (Glcβ1,6Glc)	22.2	0.45

[a] Plantain lectin.

inhibitors. *N*-acetyl-D-glucosamine also bound to the lectin whereas *N*–acetyl-D-mannosamine did not. D-arabinose was a noninhibitor whereas the ketose, D-fructose, was a good inhibitor.

Evaluating the contribution of the various hydroxyl groups of D-mannose and D-glucose for their ability to bind to the banana lectin, we found that the 2-deoxy derivative was similar in activity to free glucose, and approximately one half as active as mannose. The 3- , 4- and 6-deoxy derivatives of D-glucose were inactive at concentrations of 100 mM, illustrating the necessity of these hydroxyl groups for interaction with the lectin. The importance of the C-6 hydroxymethyl group was confirmed by the failure of methyl α- and β-D-xylopyranoside, which lack a C-6 hydroxymethyl group, to inhibit the precipitation reaction. It was also found that the 1-deoxy derivatives of glucose (1,5-anhydro-D-glucitol) and mannose (1,5-anhydro-D-mannitol), which lack glycosidic hydroxyl groups, were almost identical in their inhibitory activity to the respective free sugars.

The importance of the anomeric hydroxyl group was studied by assaying the methyl α- and β-glycosides of mannose and glucose. It was readily apparent that the methyl α-glycosides are approximately 3.5-fold more reactive than the methyl β-glycosides.

Two fluorinated sugars were assayed for their inhibitory activity. Methyl-6-deoxy-6-fluoro-α-D-glucopyranoside was a noninhibitor at a concentration of 100 mM whereas 3-deoxy-3-fluoro-D-glucose was found to be a fair inhibitor.

Of the *O*-methyl glucose derivatives assayed for their inhibitory activity, the 4-*O*- and 6-*O*-methyl derivatives were inactive at concentrations of 100 mM. Interestingly, the 2-*O*- and 3-*O*-methyl glucose derivatives inhibited to approximately the same extent. It was expected that 2-*O*-methyl glucose would be an active sugar inasmuch as glucose disaccharides linked α- and β1,2 were good inhibitors (see below), but it was surprising that 3-*O*-methyl glucose was an active inhibitor. This suggests that it may be the C-3 *O*-atom of glucose that is involved in hydrogen bonding to the protein, a hypothesis supported by the inhibitory activity of 3-deoxy-3-fluoro-D-glucose.

Next we studied a large number of glucose and mannose oligosaccharides. Kojibiose (α1,2-glucobiose), nigerose (α1,3-glucobiose), maltose, and isomaltose (α1,6-glucobiose) were all inhibitory, whereas of the β-linked analogues, cellobiose (Glc β1,4Glc) was noninhibitory at 100 mM and the β1,6-linked glucobiose, gentiobiose, was poorly inhibitory (40% inhibition at 100 mM (Table 1). Sophorose (Glc β1,2Glc), although it is β-linked, has the C-3,4 and 6 hydroxyl groups of the reducing Glc group available for interaction with the lectin; it was a fair inhibitor. Laminaribiose (Glc β1,3Glc) was a good inhibitor. Further studies of such β-glucan binding are presented in the companion paper [17]. Of the mannose disaccharides tested, there was no distinction between the α1,2-, 1,3-, and 1,6-linked oligosaccharides, all being equivalent to methyl α-mannoside. Sucrose was non inhibitory at a concentration of 100 mM.

Interestingly, maltotriose (Glcα1,4Glcα1,4Glc) was similar in its activity to maltose and panose (Glcα1,6Glcα1,4Glc) to isomaltose, suggesting that the banana lectin is interacting with a single, nonreducing α-linked D-glucosyl residue. Finally, it was observed that, although D-fructose was a fairly good inhibitor, its methyl α-furanoside was a non inhibitor; this lack of reactivity sets it apart from conA for which it is a good inhibitor. On the other hand, methyl β-fructopyranoside was a very good inhibitor; this is not surprising inasmuch as its structure is related to 1,5-anhydro-D-mannitol.

A more precise measure of binding activity was conducted by isothermal titration calorimetry. Binding of monosaccharides and α-linked dervatives were generally rather weak, with K_a values less than 1000 M^{-1} (Table 2). Because of these relatively low binding constants, concentrations of the macromolecule subunit in the range of 1–10 mM are needed in order to obtain a sigmoidal titration curve whereby a value of *n*, the number of binding sites per subunit, may be determined with reasonable certainty. Such concentrations, equivalent to a protein concentration of 14–140 mg·mL^{-1}, are not practical because of viscosity and solubility problems, titrations were therefore carried out with lectin concentrations of about 2–4 mg·mL^{-1}. At this concentration, titration curves were essentially exponential. Such curves can give reasonable values of K_a and ΔH·mol^{-1}, provided that *n* is fixed. Accordingly, all curve fitting was perfomed using a fixed value of *n* = 1. If the value of *n* is allowed to vary, K_a values are still reasonably constant over a wide range of *n* (typically 0.5–4), but ΔH·mol^{-1} values vary greatly, and nearly inversely with values of *n*. Therefore, in Table 2 we present only the K_a value, and its reciprocal, K_d (for direct comparison with I_{50} values given Table 1). Assuming that all the protein present is active lectin, and the lectin possesses one binding site per subunit, ΔH values were estimated to be in the range of −29 to −42 kJ·mol^{-1}, consistent with calorimetric studies with other lectins.

The calorimetric data are consistent with the inhibition data, although K_d values are consistently lower than I_{50} values. This is to be expected, as precipitation by poly-saccharides involves multiple interactions that require higher concentrations of monovalent inhibitors than is necessary to half-saturate binding sites of the lectin free in solution. Interestingly, but not unexpectedly, we found that Galα1,3ManαOMe was a good inhibitor of the banana lectin indicating that the lectin also recognizes mannosyl residues linked at the 3-*O*-position.

DISCUSSION

Reference to the quantitative precipitation curves of the banana lectin with a series of polysaccharides, to the carbohydrate-hapten inhibition, and to isothermal titration calorimetric data with a large number of monosaccharides, oligosaccharides and their derivatives, indicates the banana lectin to be a mannose/glucose-binding lectin. It would appear that, similar to conA [18], the banana lectin interacts with branched α-mannans (e.g. yeast mannan) and branched α-glucans (glycogen, dextrans and starches), but not linear α-glucans containing only α1,4- and α1,6-linked glucose units (e.g. isolichenan and pullulan). It also is worth noting that the banana lectin binds to Sephadex G-100, crossed linked dextran gel [1] and can be eluted with methyl α-glucoside.

A very notable difference between the banana lectin and conA is their interaction with 3-*O*-methyl glucose; the banana lectin binds to this sugar ($K_a = 1.85 \times 10^2$ M^{-1}) whereas conA does not bind [15]. This difference is reflected in the precipitation reaction of these two lectins with elsinan. As indicated above, this linear polysaccharide contains internal glucosyl residues linked α1,3. Although initially both lectins gave a precipitate with this poly-saccharide, only the banana lectin reacted with elsinan after the polysaccharide was passed through a conA-Sepharose column, thus separating out a small amount (≈ 2%) of a branched α-glucan. A further observation in support of this hypothesis is the marked precipitation curve of banana lectin with the periodate-oxidized/reduced nigeran poly-alcohol, which contains glucosyl residues linked at the 3-*O*-position by the oxidation/reduction product of 4-*O*-linked glucosyl (maltosyl) units.

A further difference in carbohydrate recognition between the banana lectin and conA is their reactivity with β-D-fructans (levans). ConA precipitated with a large number of levans, both linear and branched polysaccharides contain-ing β2,6-linked fructofuranosyl units and, in some cases, also glucose units [19]. The banana lectin reacted with only two of these polysaccharides. The basis for this recognition by conA is its ability to bind to the D-fructo-furanosyl groups present in levans [19]. This was confirmed by hapten inhibition analysis with D-furanosyl-containing sugar molecules with configuration of the hydroxyl groups at C-2, 3 and 5 identical to those at C-3, 4 and 6 of the D-gluco-/manno-pyranosyl groups. Thus, conA interacts with methyl α-D-fructofuranoside and 2,5-anhydro-D-mannitol whereas the banana lectin did not bind to either of these furanoid-containing sugars.

Yet another notable difference between the banana lectin and conA involves their interaction with the branched manno-trisaccharide, Manα1,6(Manα1,3)Man and pentasaccharide

found in the core region of N-linked glycan chains of glycoproteins. The banana lectin generated precipitation curves with both branched oligosaccharides. This may be the first instance of a trisaccharide forming a precipitate with a lectin. ConA did not precipitate with either the branched tri- or pentasaccharides although it is known to bind to the branched trisaccharide with high affinity [20–22]. Koshte and colleagues [1] established that immobilized banana lectin specifically bound Man$_{8,9}$GlcNAc$_2$, free and as their corresponding glycopeptides, a finding which is quite consistent with what we report.

It appears that the banana lectin has a very limited combining site, which accommodates a single glucosyl or mannosyl group. The fact that maltose and maltotriose, and isomaltose and panose (Glcα1,6Glcα1,4Glc) inhibited the precipitation reaction to approximately the same extent supports this contention.

The interaction of the banana lectin with internal α1,3-glucosyl is a novel observation, but is not surprising inasmuch as 3-*O*-methyl glucose bound to the lectin and 3-deoxy-3-fluoro-D-glucose was also an inhibitor. Thus, it is the *O*-atom of the C-3 hydroxyl group that likely interacts with the lectin.

In as much as 3-*O*-methyl glucose inhibits the pea and lentil lectins [16], both α2β2 mannose/glucose binding lectins, we assayed these lectins for their activity toward elsinan and nigeran polyalcohol. Neither of these lectins precipitated with those polysaccharides. This is, to the best of our knowledge, the first instance of a mannose/glucose-binding plant lectin reacting with internal α1,3-linked glucosyl groups.

Also of special interest is the interaction of banana lectin with banana starch. Figure 1B shows that, indeed, a quan-titative precipitation curve is generated. One wonders whether the lectin forms a carbohydrate-protein complex within the banana pulp. The presence of a considerable quantity (14%) of starch in bananas suggests that starch could be complexed with the lectin. The fact that including glucose or methyl α-mannoside in the extraction medium enhances the yield of lectin [1] supports this possibility.

ACKNOWLEDGEMENTS

REFERENCES

1. Koshte, V.L., van Dijk, W., van der Stelt, M.E. & Aalberse, R.C. (1990) Isolation and characterization of BanLec-I, a mannoside-binding lectin from *Musa paradisiac* (banana). *Biochem. J.* **272**, 721–726.
2. Koshte, V.L., Aalbers, M., Calkhover, P.G. & Aalberse, R.C. (1992) The potent IgG4-inducing antigen in banana is a mannose-binding lectin, BanLec-I. *Int. Arch. Allergy Immunol.* **97**, 17–24.
3. Peumans, W.J., Zhang, W., Barre, A., Houles-Astoul, C., Balint-Kurti, P.L., Rovira, P., Rouge, P., May G.D., Van Leuven, F., Truffa-Bachi, P. & Van Damme, E.J.M. (2000) Fruit-specific lectins from banana and plantain. *Planta* **211**, 546–554.
4. Peumans, W.J., Winter, H.C., Bemer, V., Van Leuven, F., Goldstein, I.J., Truffa-Bachi, P. & Van Damme, E.J.M. (1997) Isolation of a novel plant lectin with an unusual specificity from *Calystegia sepium*. *Glycoconj. J.* **14**, 259–265.

5. So, L.L. & Goldstein, I.J. (1967) Protein–carbohydrate inter-action. IV. Application of the quantitative precipitin method to polysaccharide–concanavalin A interaction. *J. Biol. Chem.* **242**, 1617–1622.

6. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

7. Goldstein, I.J., Hay, G.W., Lewis, B.A. & Smith, F. (1965) Controlled degradation of polysaccharides by periodate oxidation, reduction, and hydrolysis. *Meth. Carbohyr. Chem.* **V**. 361–370.

8. Misaki, A., Torii, M., Sawal, T. & Goldstein, I.J. (1980) Structure of the dextran of *Leuconostoc mesenteroides* B-1355. *Carbohydr. Res.* **84**, 273–285.

9. Goldstein, I.J., Poretz, R.D., So, L.L. & Yang, Y. (1968) Protein – carbohydrate interaction. XVI. The interaction of concanavalin A with dextrans from *L. mesenteroides* B-512-F, *L. mesenteroides* (Birmingham), *Streptococcus bovis*, and a synthetic alpha-(1 – 6) - D-glucan. *Arch. Biochem. Biophys.* **127**, 787–794.

10. Hehre, E.J. (1956) Natural synthesis of low molecular weight (clinical type) dextrans by a *Streptococcus* strain. *J. Biol. Chem.* **222**, 739–750.

11. Spiro, R.G. (1969) Characterization and quantitative determination of the hydroxylysine-linked carbohydrate units of several colla-gens. *J. Biol. Chem.* **244**, 602–612.

12. Goodman, J.W. & Kabat, E.A. (1960) Immunochemical studies on cross-reactions of antipneumococcal sera II. cross-reactions of types IX and XII antipneumococcal sera with dextrans. *J. Immunol.* **84**, 347–357.

13. Suzuki, H. & Hehre, E.J. (1964) The differentiation of serotype A and B dextrans by means of partial acetolysis. *Arch. Biochem. Biophys.* **104**, 305–313.

14. Tsumuraya, Y. & Misaki, A. (1977) A new fungal α-D-glucan, elsinan, elaborated by *Elsinoe leucospila. Carbohydr. Res.* **66**, 53–65.

15. Goldstein, I.J., Hollerman, C.E. & Smith, E.E. (1965) Protein–carbohydrate interactions II. inhibition studies on the interaction of concanavalin A with polysaccharides. *Biochemistry* **4**, 876–883.

16. Allen, A.K., Desai, N.N. & Neuberger, A. (1976) The purification of the glycoprotein lectin from the broad bean (*Vicia faba*) and a comparison of its properties with lectins of similar specificity. *Biochem. J.* **155**, 127–135.

17. Goldstein, I.J., Winter, H.C., Mo, H., Misaki, A., Van Damme, E.J.M. & Peumans, W.J. (2001) Carbohydrate binding properties of banana (*Musa acuminata*) lectin. II, Binding of laminaribiose oligosaccharides & β-glucans containing β1,6-glucosyl end groups. *Eur. J. Biochem.* **268**, 2616–2619.

18. Goldstein, I.J., Hollerman, C.E. & Merrick, J.M. (0000) Protein–carbohydrate interactions I. The interaction of polysaccharides with concanavalin A. *Biochim. Biophys. Acta.* **97**, 68–76.

19. So, L.L. & Goldstein, I.J. (1969) Protein–carbohydrate interaction part XXI. The interaction of concanavalin A with D-fructans. *Carbohydr. Res.* **10**, 231–244.

20. Brewer, C.F., Bhattacharyya, L., Brown, R.D. III & Koenig, S.H. (1985) Interactions of concanavalin A with a trimannosyl oligosaccharide fragment of complex and high mannose type glycoproteins. *Biochem. Biophys. Res. Commun.* **127**, 1066–1071.

21. Carver, J.P., MacKenzie, A.E. & Hardman, K.D. (1985) Molecular model for the complex between concanavalin A and a biantennary-complex class glycopeptide. *Biopolymers* **24**, 49–63.

22. Kaku, H., Goldstein, I.J. & Oscarson, S. (1991) Interactions of five D-mannose-specific lectins with a series of synthetic branched trisaccharides. *Carbohydr. Res.* **213**, 109–116.