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AN "EXTENSION" OF THE CARBOHYDRATE BINDING SPECIFICITY OF CONCANAVALIN A

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SUMMARY

Evidence based on the quantitative precipitin method and hapten inhibition technique demonstrates that concanavalin A may interact with internal 2-*O*-linked α-D-mannopyranosyl residues as may occur in glycoproteins and polysaccharides.

Concanavalin A, the sugar-binding protein of the jack bean (*Canavalia ensiformis*), has proved to be a valuable tool in many areas of the biological sciences[1]. Essential to all of its diverse biological properties is the ability of this protein to interact specifically with a select group of carbohydrate moieties[2].

By means of the quantitative precipitin method and the techniques of hapten inhibition and equilibrium dialysis it was established that the combining sites of concanavalin A are most complementary to α-D-mannopyranosyl residues but will also bind α-D-glucopyranosyl (and its 2-acetamido-2-deoxy derivative) and β-D-fructofuranosyl units[3–8]. We have proposed that unmodified hydroxyl groups at C-3, C 4 and C-6 of the D-*arabino*-hexopyranosyl configuration (Mäkelä's group 3 sugars[9]) appear to represent the minimum structural features required for saccharide binding to concanavalin A[3–6]. In addition we postulated that concanavalin A precipitates certain carbohydrate-containing macromolecules by interaction with specific glycosyl moieties situated at terminal, non-reducing oligo- and polysaccharide chain ends ("chain-end mechanism")[3–8,10,11].

It now appears necessary to modify our original concept of an exclusive "chain-end mechanism" to account for all concanavalin A–carbohydrate interactions. The experimental observation which provided the first exception to our hypothesis was the finding that sophorose (2-*O*-β-D-glucopyranosyl-D-glucose) inhibited concanavalin A–dextran interaction[12]. All our previous studies had shown that only α-linked D-glucose- and D-mannose-containing disaccharides bind to concanavalin A. This was the first indication that internal sugar residues could bind to the protein, for it was shown unequivocally that concanavalin A binds to the reducing D-glucosido moiety of this β-glucobiose.

Some years ago, Hehre[13,14] and Suzuki and Hehre[15] raised the same question when they pointed out that 2-*O*-substituted α-D-glucopyranosyl residues which occur in many dextrans possess the configurational features which we suggested were necessary for interaction with concanavalin A (unmodified hydroxyl groups at C-3, C-4 and C-6 of α-D-glucopyranosyl residues).

Furthermore, it has been observed that certain glycopeptides lacking terminal α-D-glucopyranosyl or α-D-mannopyranosyl residues inhibit erythrocyte hemagglutination[16] by concanavalin A. Similar studies which implicate internal residues of cell receptor glycoproteins have been conducted by Kornfeld and his colleagues[17] on phytohemagglutinins from *Lens culinaris*, *Agaricus bisporus*[18] and *Robinia pseudoaccacia*[19].

In this paper we provide the evidence which demonstrates that concanavalin A may interact with internal (1→2)-linked α-D-mannopyranosyl residues.

Concanavalin A was prepared by the method of Agrawal and Goldstein[20]. The procedures for quantitative hapten inhibition[3] and agar gel diffusion[21] have been described previously. Quantitative precipitin analyses were performed as previously reported[8] with the exception of smaller reaction volumes (1.0 ml) and a longer incubation period (1 week). Oligosaccharides were isolated as described[22,23] except for 2-*O*-β-D-glucopyranosyl-D-mannose which was synthesized in this laboratory by a procedure which will be described elsewhere. Methyl α-D-glucopyranoside and methyl α-D-mannopyranoside were purchased from Pfanstiel Laboratories, Waukegan, Illinois. *Klebsiella* K-24 (ref. 24) was obtained from Prof. Guy Dutton, University of Vancouver, Canada; *Klebsiella* K-57 (ref. 25) from Prof. Bengt Lindberg, University of Stockholm, Sweden and *Klebsiella* K-11 from Dr S. Stirm, Max-Planck Institut für Immunbiologie, Freiburg, W. Germany.

Both of the trisaccharides shown in Fig. 1 are good inhibitors of the concanavalin A–dextran B-1355-S precipitation reaction despite the fact that neither possesses a nonreducing α-D-mannopyranosyl terminus. Inasmuch as D-galactose and its derivatives do not bind to concanavalin A (refs 3–6) we may infer that 2-*O*-substituted α-D-mannopyranosyl residues can bind to the active sites of the protein, a not so surprising conclusion when it is recalled that the C-3, C-4 and C-6 hydroxyl groups are still available for interaction. It may also be noted that 2-*O*-methyl-D-mannose was shown to be equivalent to D-mannose as an inhibitor of the concanavalin A system[6].

Borohydride reduction of trisaccharide A [α-D-Gal-*P*(1→2)-α-D-Man-*P*(1→2)-D-Man], which contains two α-(1→2)-linked D-mannose units, affords the corresponding trisaccharide alditol. The latter still contains an internal α-(1→2)-D-mannopyranosyl unit and, although less potent than the parent trisaccharide A, nevertheless inhibits to the same extent as methyl α-D-mannopyranoside (Fig. 1).

The two β-linked disaccharides (2-*O*-β-D-mannopyranosyl-D-mannose and 2-*O*-β-D-glucopyranosyl-D-mannose) inhibited concanavalin A–dextran interaction to a much lesser extent than methyl α-D-mannopyranoside and 2-*O*-α-D-mannopyranosyl-D-mannose: the latter disaccharide contains two D-mannosyl units each of which is potentially capable of binding to concanavalin A (*cf.* ref. 26).

An alternate approach to assessing the potential of 2-*O*-substituted α-D-mannopyranosyl residues as receptor sites for concanavalin A involves precipitation studies with macromolecules of known constitution. The repeating units of the polysaccharides of several *Klebsiella* species have been elucidated. Some of these polysaccharides

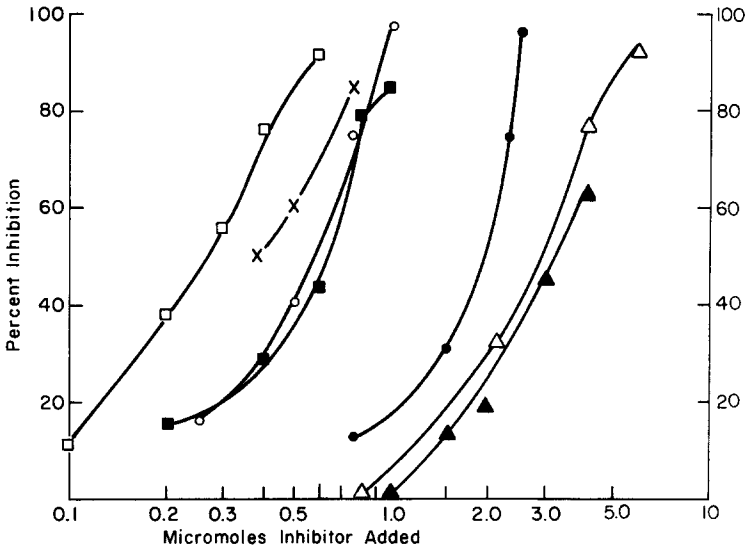
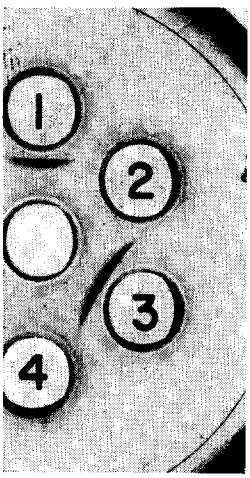


Fig. 1. Inhibition by saccharides of concanavalin A–dextran B-1355-S interaction. Each tube contained concanavalin A (340 μg), dextran B-1355-S (600 μg) and inhibitor as noted in a total volume of 3.0 ml. □, *O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannose (trisaccharide A); ×, *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-mannopyranosyl-(1→2)-D-mannose; ○, methyl α-D-mannopyranoside; ■, *O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannitol (reduced trisaccharide A); ●, methyl α-D-glycopyranoside; △, 2-*O*-β-D-glucopyranosyl-D-mannose; ▲, 2-*O*-β-D-mannopyranosyl-D-mannose.

possess internal 2-*O*-linked α-D-mannopyranosyl units as the only saccharide capable of binding to concanavalin A. In several instances we have been able to demonstrate reactivity of concanavalin A with certain *Klebsiella* polysaccharides. These include K-24 and K-57. (It is of great interest that K-24, reported to contain one *O*-acetyl group[24] did not give a precipitate with concanavalin A until we had treated it with 0.1 M aq. NaOH.) Fig. 2 presents the photograph of an Ouchterlony two dimensional agar gel diffusion plate showing the reaction of *Klebsiella* K-24, K-11 and K-57. As expected, the polysaccharide from *Klebsiella* K-11 which contains neither D-mannose nor D-glucose failed to react with concanavalin A.

Fig. 3 shows the precipitin curve generated when a constant amount of concanavalin A (46 μg N) interacts with increasing amounts of K-24. The inset in Fig. 3 shows the solubility of the concanavalin A–polysaccharide complex. The solubility of the concanavalin A–K-24 precipitate is very high (15 μg N/ml). This taken together with the great ease with which methyl α-D-mannopyranoside inhibits the concanavalin A–K-24 precipitation reaction (0.036 μmole glycoside for 50% inhibition compared to 0.6 μmole for the concanavalin A–dextran B-1355-S system[4]) leads us to suggest that the binding of concanavalin A to the isolated 2-*O*-linked α-D-mannopyranosyl residues in K-24 may be quite weak.

These experiments suggest that 2-*O*-linked α-D-mannopyranosyl residues (and almost certainly 2-*O*-linked α-D-glycopyranosyl units), when accessible, may serve as receptor sites for concanavalin A. Furthermore, when α-(1→2)-linked α-D-mannopyranosyl residues occur in sequence they appear to be more effective as concanavalin A receptors than when they occur in isolation (*cf.* ref. 26).



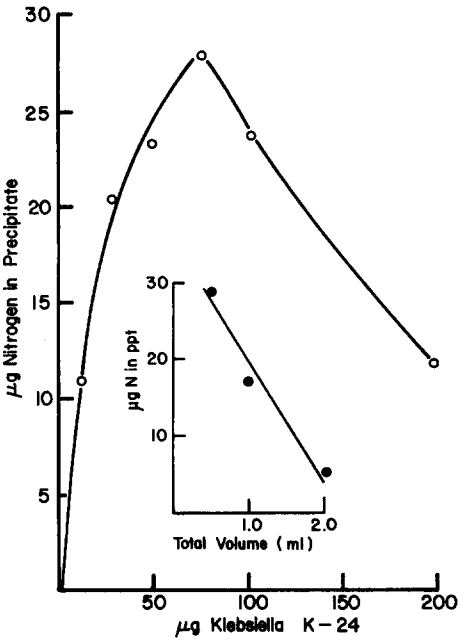


Fig. 2. Two-dimensional agar gel diffusion patterns. Central well: concanavalin A (4 mg/ml). Well 1, *Klebsiella* K-24 (1.0 mg/ml); 2, *Klebsiella* K-11 (1.0 mg/ml); 3, *Klebsiella* K-57 (0.66 mg/ml); 4, saline control.

Fig. 3. Quantitative precipitation curve of *Klebsiella* K-24 polysaccharide with concanavalin A. Concanavalin A, 46 μg of nitrogen per tube. Inset shows the effect of volume on concanavalin A–*Klebsiella* K-24 polysaccharide precipitation. Concanavalin A, 46 μg of nitrogen; *Klebsiella* K-24 polysaccharide, 75 μg.

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