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D-Glucuronic acid: A non-inhibitor of the concanavalin A system

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SUMMARY

D-Glucuronic acid is demonstrated not to inhibit the concanavalin A system; previous claims to the contrary are shown to be due to a pH effect. Concanavalin A does not precipitate biopolymers containing nonreducing, terminal α-D-glucopyranosiduronic acid residues.

The current use of concanavalin A, the phytohemagglutinin of the jack bean, as a probe for investigating membranous glycoproteins and glycolipids requires a detailed knowledge of the protein's specificity. Extensive hapten inhibition studies employing mono- and oligosaccharides and their simple derivatives demonstrated that the concanavalin A combining sites are complimentary to α-D-mannopyranosyl and α-D-glucopyranosyl (or its 2-acetamido-2-deoxy derivative) residues with a specific requirement that the hydroxyl groups at the C-3, 4 and 6 positions of these sugars be unmodified.

In a recent communication it was reported that glucuronic acid is a better inhibitor than D-glucose of concanavalin A—polysaccharide interaction. If this is true, it represents an important extension of the sugar-binding specificity of this protein. Since we had already shown that an unmodified C-6 hydroxymethyl group is an important concanavalin A binding locus we have reinvestigated the claim that glucuronic acid can serve as a substrate for concanavalin A. We report here our findings that low concentrations of glucuronic acid are noninhibitory, and that at higher concentrations the inhibitory action of glucuronic acid is nonspecific and can be explained by a depression of the pH of the incubation medium to a point where precipitation does not occur. The finding that D-glucuronic acid does not interact with concanavalin A gains added significance in the light of reports that certain charged, glucuronic acid-containing mucopolysaccharides form specific insoluble precipitates with concanavalin A.

Concanavalin A was prepared by the method of Agrawal and Goldstein\textsuperscript{10}. Quantitative precipitin studies using dextran B-1355-S were conducted as described previously\textsuperscript{11}. Turbidimetric assays were done by the method of Goldstein et al.\textsuperscript{2} using glycogen and dextran B-1355-S and did not differ significantly from that of Plow and Resnick\textsuperscript{6}. D-Glucuronolactone was a product of Mann Research Labs, Orangeburg, N.Y. D-Glucose, D-galactose, methyl α-D-glucopyranoside and glycogen were purchased from Pfannstiehl Labs, Waukegan, Ill. Methyl α-D-glucopyranosiduronic acid, prepared from methyl α-D-glucopyranoside by the method of Barker et al.\textsuperscript{12}, was purified by chromatography on a Dowex 1-X8 (CH\textsubscript{3}COO\textsuperscript{−}) ion-exchange column\textsuperscript{13}. The acid, shown to be free of methyl α-D-glucopyranoside by thin-layer chromatography, assayed for 93% purity (on a weight basis) with the carbazole reagent\textsuperscript{14}. Native dextran N-4 (elaborated by \textit{Leuconostoc mesenteroides} Strain N-4) was from the Meito Industrial Co., Japan. Catalytic oxidation of dextran N-4 was conducted by the procedure of Aspinall and Nicolson\textsuperscript{15}. An extracellular, branched polysaccharide isolated from the culture medium of \textit{Aerobacter} sp. IFO 1236\textsuperscript{9} was shown to consist of D-galactose, D-glucuronic acid and D-mannose, the glucuronic acid residues occurring principally as α-linked nonreducing termini. Reduction of the D-glucuronic acid residues was conducted by the method of Sutherland\textsuperscript{16}. Three such treatments resulted in the conversion of all D-glucuronic acid residues into D-glucosyl units as determined by paper and gas–liquid chromatography. (Molar ratios D-galactose: D-glucose:D-mannose, 3.2:1.08:1.0.)

\begin{table}
\caption{Hapten Inhibition Analysis of the Concanavalin A–Glycogen Precipitation Reaction}
\centering
\begin{tabular}{lcc}
\hline
\textbf{Inhibitor} & \textbf{Concen} & \textbf{Absorbance} & \textbf{pH} \\
 & (\textmu M) & \textbf{at 420 nm} & \\
\hline
 &  & 0.350 & 6.25 \\
D-Glucuronolactone & 9 & 0.360 & 6.25 \[0.5em]
 & 20 & 0.335 & 6.18 \\
 & 30 & 0.380 & 6.30 \\
D-Galactose & 200 & 0.380 & \\
 & 300 & 0.370 & \\
Methyl α-D-glucopyranoside & 2 & 0.320 & \\
 & 4 & 0.290 & \\
 & 10 & 0.170 & \\
 & & 0.0 & \\
\hline
\end{tabular}
\end{table}

Using the same experimental conditions described by Plow and Resnick\textsuperscript{6} we obtained the data shown in Table I. It will be noted that glucuronolactone does not inhibit the precipitation reaction between concanavalin A and glycogen. Essentially the same results were obtained whether the glucuronolactone was freshly prepared or allowed to stand in solution overnight, the pH of the incubation mixture not changing significantly. When increased concentrations of D-glucuronolactone were tested it was found that the pH of the incubation mixture decreased with a concomitant decrease in precipitation. Thus, using 40, 100, 300 and 500 μmoles of the lactone it was observed that no precipitation occurred with the last two concentrations of lactone, the respective pH values in each case being 5.9, 5.4, 3.8, and 3.7. Contrary to the report of Plow and Resnick\textsuperscript{6}, D-galactose (300 μmoles) failed to inhibit the precipitation reaction whereas methyl α-D-glucopyranoside proved to be a good inhibitor\textsuperscript{2−5}.

Synthetic methyl α-D-glucopyranosiduronic acid (28 μmoles) inhibited completely the interaction of concanavalin A with dextran B-1355-S. However, the pH of the incubation mixture was found to be 3.65, below the pH at which precipitation occurs\textsuperscript{7}. Adjusting the pH of a solution of methyl α-D-glucopyranosiduronic acid to pH 7.0 gave control amounts of turbidity demonstrating the inhibition was, in reality, a pH effect.

The results of quantitative precipitin inhibition analysis of the concanavalin A—dextran system are presented in Fig. 1. D-Glucose and methyl α-D-glucopyranoside inhibited precipitation whereas D-galactose (300 μmoles) failed to inhibit the precipitation reaction whereas methyl α-D-glucopyranoside proved to be a good inhibitor\textsuperscript{2−5}.

![Fig. 1. Inhibition by saccharides of dextran-concanavalin A precipitation. Each tube contained concanavalin A (46.5 μg nitrogen), dextran B-1355-S (140 μg) and inhibitor as noted in total volume of 1.0 ml. The final reaction mixture was 1 M in NaCl and 0.018 M with respect to phosphate buffer (pH 7.2). ○, D-glucose; •, methyl α-D-glucopyranoside; ◯, D-glucuronolactone; ●, methyl α-D-glucopyranosiduronic acid.](image)

![Fig. 2. Quantitative precipitin curves of concanavalin A with dextran N-4 (○) and its oxidized product (●). Each tube contained concanavalin A (157 μg nitrogen) in a total volume of 1.0 ml. Full procedure described by So and Goldstein\textsuperscript{7}.](image)
inhibit the concanavalin A-dextran system as described previously\(^2\text{-}^5\): D-galactose did not inhibit at a level of 400 \(\mu\)moles, also as previously noted\(^2\text{-}^5\). D-Glucuronolactone at levels of 40–190 \(\mu\)moles (solutions adjusted to pH 7) is noninhibitory. Methyl \(\alpha\)-D-glucopyranosiduronic acid at concentrations of 20–197 \(\mu\)moles (solutions adjusted to pH 7) also failed to inhibit the concanavalin A system. These inhibition experiments demonstrate unequivocally that D-glucuronic acid is not an inhibitor of the concanavalin A system. The following two experiments further substantiate this conclusion.

Dextran N-4, similar in structure to dextran B-512 (ref. 17), gave the quantitative precipitin curve shown in Fig. 2. Catalytic oxidation\(^1\) of this dextran results in the transformation of nonreducing \(\alpha\)-D-glucopyranosyl residue to \(\alpha\)-D-glucopyranosiduronic acid units. A neutralized solution of the oxidized dextran N-4 failed completely to precipitate concanavalin A (Fig. 2).

In a second experiment, it was found that the extracellular, branched polysaccharide\(^1\) from \textit{Aerobacter} sp. IFO 12369, containing multiple nonreducing \(\alpha\)-D-glucopyranosiduronic acid termini, did not precipitate with concanavalin A. However, the carboxyl reduced polysaccharide\(^1\), containing nonreducing, terminal \(\alpha\)-D-glucopyranosyl residues, gave a typical precipitin reaction with concanavalin A. Thus, concanavalin A can serve the useful function of detecting terminal, nonreducing \(\alpha\)-D-glucopyranosiduronic acid residues after reduction to corresponding \(\alpha\)-D-glucopyranosyl units.

The present data prove that a carboxyl group at the C-6 position destroys the capacity of D-glucose to inhibit the concanavalin A system. In order to diminish the difficulty of interpretation in the case of D-glucuronic acid or its lactone, both capable of existing in several different structural forms, we synthesized methyl \(\alpha\)-D-glucopyranosiduronic acid. This sugar acid possesses a fixed \(\alpha\)-D-pyranosidic ring structure, the form which would most likely bind to concanavalin A. At pH 7, this sugar failed to react with concanavalin A at a concentration level 8 times higher than that giving 50% inhibition with D-glucose. On the other hand, high concentrations of unneutralized, acidic solutions of D-glucuronic acid (or its \(\alpha\)-methyl glycoside) do indeed inhibit the precipitation reaction between concanavalin A and glycogen or dextran. But this result can be explained on the basis of a pH effect: concanavalin A precipitates biopolymers optimally between pH 6.1 and 7.2; below pH 4.7 and above pH 9.1, precipitate formation does not occur\(^7\).

With reference to reports\(^8\),\(^9\) that certain charged polysaccharides form a precipitate with concanavalin A, we suggest that glucuronic acid residues cannot play the role of a receptor site for concanavalin A. At least one report of heparin acting as a precipitant of concanavalin A has been attributed to possible contamination by a glycan impurity\(^9\). In fact, many reported instances of specific interaction of charged biopolymers with concanavalin A could be due to charge-charge interaction.

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REFERENCES
