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Effects of hydroxyl compounds on the interaction of Concanavalin A with polysaccharides*

The phytohemagglutinin Concanavalin A, isolated from jack bean meal by SUMNER AND HOWELL[1], has been shown to react with a variety of biopolymers. The precipitin reaction has been demonstrated with both neutral and charged polysaccharides, as well as with nucleic acids and immunoglobulins[2-8]. While insoluble complexes are formed with high molecular weight compounds, the binding of low molecular weight polyhydroxy substances results in a soluble complex. Furthermore, many of the low molecular weight compounds, e.g. monosaccharides and oligosaccharides, are known to competitively inhibit the binding of Concanavalin A to polysaccharides.

While the exact mechanism of interaction is unknown, certain stereochemical considerations have already been elucidated. For example, Concanavalin A reacts preferentially with certain unmodified hydroxyl groups present on terminal mannopyranosyl and glucopyranosyl residues found in branched polysaccharides[5,9]. At the same time, Concanavalin A also reacts with a variety of polyelectrolytes, none of which have the requisite nonreducing ends[8]. Perturbation studies of the globulin in the presence of D-glucose indicated a number of inflection points at different wavelengths; however, corresponding optical rotatory dispersion studies could not detect any conformational changes in Concanavalin A (ref. 10).

Temperature studies and the use of protein denaturants suggest the possibility that hydrogen bonding is involved in complex formation[11]. However, while hydrogen bonding as well as electrostatic forces have been implicated, their presence alone does not explain the earlier observation that many neutral low molecular weight polyhydroxy compounds were found to be noninhibitors[5].

The purpose of this report is to present data which indicate that the binding of Concanavalin A to neutral polyhydroxy compounds simply represents a form of hydrogen bonding with steric considerations for compounds of higher molecular weight. Furthermore, during the course of this investigation, some of the compounds previously thought to be noninhibitory were actually found to inhibit while still others enhanced the binding of Concanavalin A to neutral polysaccharides.

Concanavalin A was prepared according to the method of AGRAWAL AND GOLD-STEIN[12]. The isolated globulin was extensively dialyzed against cold distilled water, lyophilized and stored at −20° until ready for use. A stock solution containing 1.1 mg/ml in 0.25 M NaCl was prepared and used throughout this study. The turbidimetric assay system consisted of the following: 1.0 ml of a 0.054 M phosphate buffer (pH 7.0), 1.0 ml of 4.0 M NaCl, 0.6 ml of a rabbit glycogen (Mann Research Laboratories, Orangeburg, N.Y.**) solution (2 mg/ml in H_2O), 0.5 ml of the Concanavalin A stock solution, and varying quantities of inhibitor and water for a total volume of 6.2 ml. The reaction was initiated by the addition of Concanavalin A; the mixtures were then shaken, incubated for 10 min at room temperature and read at 420 nm
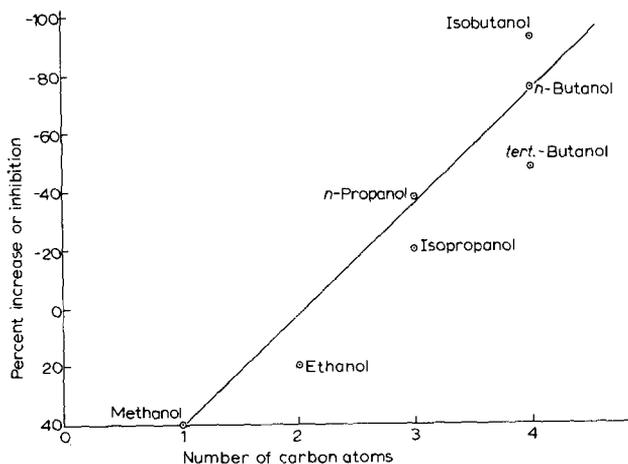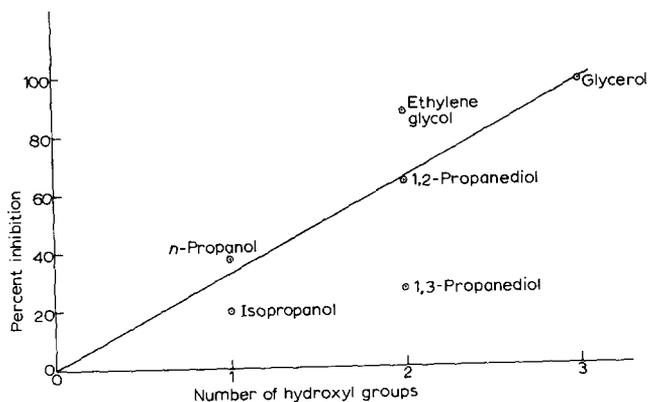

Fig. 1a. Relation between number of OH-groups in a three-carbon chain and % inhibition. The isomers are included for comparative purposes. Results were obtained with 8.0 mmoles of each compound tested. Experimental details are described in text.

Fig. 1b. Relation between carbon skeleton in primary alcohols and % increase or decrease in binding of Concanavalin A to glycogen. Secondary and tertiary alcohols are included for comparative purposes. Results were obtained with 8.0 mmoles of each compound tested. Experimental details are described in text.

using a 1.0 cm pathlength cell in a Gilford Spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio*). The blank solutions were prepared in a similar manner but contained no Concanavalin A. The % inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_1 - A_2}{A_1} \times 100,$$

where A_1 = absorbance of system without inhibitor, and A_2 = absorbance of system with inhibitor.

* See footnote on p. 657.

In Figs. 1a and 1b we present the effects of a series of compounds on the binding of Concanavalin A to glycogen. For Fig. 1a the effect of increasing numbers of hydroxyl groups on a three-carbon chain, *versus* the % inhibition is plotted. For further comparisons, the effects of two isomers, *viz.*, isopropanol and 1,3-propanediol, as well as the dihydroxy, two-carbon compound, ethylene glycol, are also included. It is to be noted that when glycerol, containing three hydroxyl groups is used, 100% inhibition is obtained; in fact, only 2.5 mmoles of glycerol are required for 50% inhibition (see Table I). Increasing the number of hydroxyl groups in a linear carbon chain requires only μmole quantities for complete inhibition (see Table I). Furthermore, from Fig. 1a it would appear that the % inhibition of the Concanavalin A–glycogen precipitin reaction is directly proportional to the number of hydroxyl groups on the carbon skeleton; increasing the number of hydroxyl groups increases the extent of hydrogen bonding and thereby decreases the affinity of Concanavalin A for the polysaccharide. The steric effects, however, must also be considered as evidenced by the results obtained with the isomers isopropanol and 1,3-propanediol. Of further interest is the fact that the dihydroxy, two-carbon compound, ethylene glycol, showed greater inhibition than 1,2-propanediol or 1,3-propanediol. This would indicate that an increased carbon chain gives rise to steric effects. Stereochemical considerations would also seem to be indicated by the fact that adjacent hydroxyl groups have a greater inhibitory capacity; the order of inhibitory effects for the dihydroxy compounds was found to be, ethylene glycol > 1,2-propanediol > 1,3-

TABLE I

THE CONCENTRATION REQUIREMENTS OF POLYHYDROXY COMPOUNDS FOR 50% INHIBITION

The assay system for the binding of Concanavalin A to glycogen was carried out in the presence of varying amounts for each compound listed. The concentration requirement for 50% inhibition was determined by first establishing, for each compound, the amount required for complete inhibition of the binding of Concanavalin A to glycogen. Total volume of assay system was 6.2 ml.

Compound	mmoles required for 50% inhibition
Methyl-α-D-glucoside	0.00124
Glucuronic acid	0.0145
D-Glucose	0.0172
D-Galactose	0.18
D-Arabinose	0.22
Sorbitol	0.68
Mannitol	1.20
D-Ribose	1.20
Dulcitol	1.28
L-Arabinose	2.30
L-Arabitol	2.50
Glycerol	2.50
Ethylene glycol	3.82
Isobutanol	4.00
n-Butanol	5.25
1,2-Propanediol	6.40
tert.-Butanol	8.30
n-Propanol	10.22
1,3-Propanediol	11.45
Isopropanol	12.80

propanediol. The effect of chain length was, therefore, investigated as shown in Fig. 1b.

The effect of a series of primary alcohols with increasing chain length, from one to four carbons, is plotted in Fig. 1b; the isomers isopropanol, isobutanol, *tert.*-butanol are also included for comparison. The surprising feature here is that both methanol and ethanol increase the "effective" binding of Concanavalin A to glycogen. Both alcohols, in the concentrations used, showed no effect by themselves on the turbidimetric assay system when incubated for varying lengths of time (up to 30 min) in the presence of either protein or polysaccharide alone. The addition of the missing component, followed by the 10-min incubation period, always gave identical results. Furthermore, no protein conformational changes, due to the presence of the alcohols, could be detected by spectrophotometric perturbation studies.

The apparent increase in the binding capacity of Concanavalin A to glycogen in the presence of both methanol and ethanol can be explained by the enhancement of the precipitin reaction by these alcohols. A similar effect for both methanol and ethanol has been demonstrated for antigen–antibody complexes[13]. However, in Fig. 1b, a linear relationship appears to hold up to *n*-butanol (higher alcohols could not be used due to limiting solubilities). Steric effects are also evident by comparison with various isomers, *viz.*, isopropanol, isobutanol and *tert.*-butanol. The strong influence of chain length on the effectiveness of straight chain alcohols in the binding capacity of Concanavalin A to glycogen, suggests the involvement of hydrophobic interactions. This may occur through the formation of competitive hydrophobic bonds between the alcohol and nonpolar residues of the protein. However, it is also possible that the aliphatic chain bound to the protein through its hydroxyl function could sterically block adjacent hydrogen bonding sites. That hydrogen bonding is of primary importance is indicated by the fact that an increase in the number of hydroxyl groups increases the effectiveness of the compound to inhibit the binding of Concanavalin A to glycogen. This is quite apparent from the list of compounds shown in Table I, where increase in the number of hydrophilic groups in a compound increases its effectiveness as an inhibitor.

In Table I, the concentration requirements for 50% inhibition of a number of polyhydroxy compounds are listed. Some of these compounds were previously believed to be noninhibitory; this discrepancy seemed to reflect differences in the concentrations used in earlier studies[5]. For further comparison, the value obtained with methyl-α-D-glucoside is included; our value of 1.24 μmoles required for 50% inhibition compares favorably with the value of 1.20 μmoles obtained by previous investigators. Further examination of Table I indicates that while arrangements of the hydroxyl groups in the compound does influence the concentration requirements for inhibition to occur, it certainly is not the sole determinant for binding these low molecular weight compounds to Concanavalin A. The criteria for binding appear to be more "basic" and show a dependence on the number of hydroxyl groups present on the inhibitor with stereochemical considerations influencing the potency of binding. Also, it is interesting to note that conversion of a hydroxyl group to an aldehydic or carboxyl function further enhances the inhibitory capacity of the compound.

While the results reported in this study were obtained with a glycogen preparation, similar results were also obtained using a yeast mannan (Pierce Chemical

Company, Rockford, Ill.*) preparation as the polysaccharide. Furthermore, when an assay system at a pH of 5.5 (acetate buffer) was used, the same order of inhibition as is shown in Table I was obtained.

From the wide range of compounds capable of interacting with Concanavalin A, it appears that the forces which bind molecules to this protein are less specific than those which bind haptens to antibodies and substrates to enzymes. The relationship between hydroxyl groups and binding indicates that these forces are hydrophilic in nature and thus suggests the involvement of hydrogen bonding.

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* See footnote on p. 657.