The binding of concanavalin A to various structures via hydrophobic interactions has been studied using a variety of physicochemical assays. It was found that concanavalin A binds to nonpolar compounds such as the plant auxin β-indoleacetic acid and its structural analogue tryptophan and that this binding is independent of the saccharide-binding activity normally associated with the lectin. The results of equilibrium dialysis experiments on the binding of β-indoleacetic acid were consistent with the presence of a single weak binding site per subunit of protein, having an association constant of about $7 \times 10^4$ M$^{-1}$. Competition experiments using various nonpolar compounds such as o-iodobenzoic acid suggested that this hydrophobic binding site is located in the same cavity which binds the iodine-containing ligand as shown by x-ray crystallography. Concanavalin A also binds to lipid vesicles composed of dipalmitoylphosphatidylcholine or 12:0-tetradecanoyl phorbol-13-O-acetate. This binding to lipid membranes raises the possibility that the synergistic effects of concanavalin A and tetradecanoyl phorbol acetate on lymphocyte mitogenesis may be due in part to an interaction between lectin and the phorbol ester.

Studies on the structure and activities of the lectin concanavalin A (Con A) have been pursued in a number of laboratories with the goal of understanding the wide variety of biological and ligand-binding activities exhibited by this plant protein. Con A$^1$ at physiological pH has been shown to be a tetramer of identical subunits (3, 4), each with a single weak binding site per subunit of protein, having an association constant of about $7 \times 10^4$ M$^{-1}$. Competition experiments using various nonpolar compounds such as o-iodophenol, o-iodobenzoic acid, and o-iodophenyl-β-D-glucoside, as well as o-iodophenyl-β-D-galactoside, all bind to crystalline Con A in a pocket of each subunit that contains many hydrophobic amino acids (4, 10, 11). In agreement with these findings, preliminary nuclear magnetic resonance experiments using o-iodophenyl-β-D-glucoside uniformly labeled with $^3$H in the sugar moiety have suggested that this aryl saccharide binds to a secondary site on Con A in addition to the saccharide-binding site (12). All of these results are consistent with the presence of a second binding site on the Con A protomer that is capable of interacting with hydrophobic ligands. This raises the possibility that Con A may interact with the cell membrane via a hydrophobic portion of the molecular surface that alone would not bind; this secondary interaction may provide the surface alterations necessary for some of the lectin's biological effects.

In order to explore this secondary interaction hypothesis further, we have characterized the binding of Con A in solution to a number of ligands that do not contain any saccharide moiety in their structures. It has been shown, however, that Con A also binds to various molecules that do not contain any saccharide moiety in their structures. It has been shown, for example, that Con A will bind and fuse lipid vesicles devoid of any carbohydrate group (7, 8). It has also been reported that chromatography of interferon on Sepharose columns derivatized with Con A results in the binding of the interferon; this complex can be dissociated only by adding to the buffer compounds such as ethylene glycol, which reduces the polarity of the solvent (9). Furthermore, x-ray crystallographic experiments have shown that compounds containing nonpolar groups, such as o-iodophenol, o-iodobenzoic acid, and o-iodophenyl-β-D-glucoside, as well as o-iodophenyl-β-D-galactoside, all bind to crystalline Con A in a pocket of each subunit that contains many hydrophobic amino acids (4, 10, 11). In agreement with these findings, preliminary nuclear magnetic resonance experiments using o-iodophenyl-β-D-glucoside uniformly labeled with $^3$H in the sugar moiety have suggested that this aryl saccharide binds to a secondary site on Con A in addition to the saccharide-binding site (12). All of these results are consistent with the presence of a second binding site on the Con A protomer that is capable of interacting with hydrophobic ligands. This raises the possibility that Con A may interact with the cell membrane via a hydrophobic portion of the molecular surface that alone would not bind; this secondary interaction may provide the surface alterations necessary for some of the lectin's biological effects.

In order to explore this secondary interaction hypothesis further, we have characterized the binding of Con A in solution to a number of ligands that do not contain any saccharide moiety in their structures. In the present communication, we report the binding of Con A to several hydrophobic structures, most notably the plant auxin β-indoleacetic acid. We have also examined the effect on lymphocyte mitogenesis when Con A and another mitogen that is known to be hydrophobic interact at the cell surface.

**EXPERIMENTAL PROCEDURES**

Materials — The following reagents were obtained commercially: tryptophan (Sigma); β-indoleacetic acid (Aldrich); and 12:0-tetradecanoyl phorbol-13-O-acetate (TPA) (Consolidated Midland Corp.). All other chemicals employed in this study were reagent grade and, unless otherwise specified, were used without further purification. $[^3]$H[Tryptophan (8 Ci/mmol), β-indole$[^3]$C]acetate (50 mCi/mmol), $[^3]$Hgalactose (6 Ci/mmol), and $[^3]$Hcolchicine (3 Ci/mmol) were obtained from New England Nuclear. α-methyl-D-$[^3]$C]glucoside
(MeGlc) (52 mCi/mmol) was from Calhotics. TPA (40 mg) was labeled with \(^{3}H\) (18 mCi) by Biochemical and Nuclear Corp. using the postlabeling discharge activation procedure (12). The labeled mixture was purified by thin-layer chromatography on Brinkmann precoated Silica Gel-25 plates using the solvent system of ethyl acetate and benzene (1:1 v/v). The material migrating at the position corresponding to unlabeled TPA \((R_f = 0.46)\) was eluted with two washes of acetone, dried, and redissolved in dimethyl sulfoxide prior to use. The specific activity of this preparation of \(^{3}H\)TPA was 1.42 \(\times 10^{4}\) cpm/mmol.

Con A, succinyl-Con A, and \(^{3}H\)Con A were prepared as described previously (14, 15). Procedures for the isolation of the other lectins, phytohemagglutinin, wheat germ agglutinin, Favs1, and green pea lectin, have also been reported (16-18). Bovine serum albumin and \(\beta\)-lactoglobulin were from Pentex.

Methods—Agarose-\(\alpha\)-aminoacylpropyl-tryptophan methyl ester was purchased from Miles Laboratories and \(\alpha\)-aminoacylpropyl-\(H\) TPA. A similar procedure was used to study the interaction between \(\alpha\)-aminoacylpropyl-\(H\) TPA and Con A with a control buffer. The materials in Component A (Fig. 1) were eluted with the protein in the void volume (Fig. 2a). These results suggest that the binding of tryptophan to Con A resulted in the appearance of protein-associated radioactivity, well separated from the bulk of the free labeled amino acid.

Control experiments were performed to test the reliability of this assay system for detecting the binding of radioactive ligands to proteins. The experiments were based on results that had been previously characterized by other physicochemical methods: (a) bovine serum albumin binds to tryptophan (23); (b) Con A binds to MeGlc but not to D-galactose (6); and (c) Con A does not bind to colchicine (24). In each of these cases, the predicted profile of the radioactive low molecular weight ligand was observed (Fig. 2c to f). All of these results suggested that the chromatographic system could serve as a quick and reliable test for the binding of radioactive ligands to Con A and that the presence of radioactive tryptophan in the Con A-containing fractions of the assay column indicates the presence of radioactive derived at 

![Fig. 1](http://www.jbc.org/)

**Fig. 1.** Chromatography of Con A (7 mg/ml, 1.5 ml) on a column of agarose derivatized with \(\alpha\)-aminoacylpropyl-tryptophan methyl ester. The majority of the protein was eluted with a skewed absorbance profile (Fig. 1). In contrast, chromatography of Con A on a column of underivatized agarose yielded a single symmetrical peak. This suggested that the skewed elution profile may have been due to weak interactions between the protein and the column material (Fig. 1). After extensive washing, a small but reproducible amount of protein (\(\sim 0.1\) mg) could be further eluted using buffer containing 50% (v/v) ethylene glycol to reduce the polarity of the solvent. The eluted material (Component B, Fig. 1) was shown by polyacrylamide gel electrophoresis to contain the intact subunit of Con A, indistinguishable from the material in Component A (Fig. 1). Similar results were obtained using \(\alpha\)-aminoacylpropyl covalently coupled to Affi-Gel 10 (N-hydroxysuccinimide ester of succinylated anaprolein Bio-Gel A).

In order to test for direct interaction between the Con A and tryptophan molecules, a chromatographic assay on Bio-Gel P-60 was performed using \(^{3}H\)tryptophan as the ligand. When \(^{3}H\)tryptophan was incubated with Con A and subjected to column chromatography, about 0.3% of the total radioactivity was eluted with the protein in the void volume (Fig. 2a). In contrast, no radioactivity was observed in the fractions emerging in the void volume of the column when \(^{3}H\)tryptophan alone was chromatographed (Fig. 2b). These results suggest that the binding of tryptophan to Con A resulted in the appearance of protein-associated radioactivity, well separated from the bulk of the free labeled amino acid.

Control experiments were performed to test the reliability of this assay system. For detecting the binding of radioactive ligands to proteins. The experiments were based on results that had been previously characterized by other physicochemical methods: (a) bovine serum albumin binds to tryptophan (23); (b) Con A binds to MeGlc but not to D-galactose (6); and (c) Con A does not bind to colchicine (24). In each of these cases, the predicted profile of the radioactive low molecular weight ligand was observed (Fig. 2c to f). All of these results suggested that the chromatographic system could serve as a quick and reliable method for the binding of radioactive ligands to Con A and that the presence of radioactive tryptophan in the Con A-containing fractions of the assay column indicates ...
Hydrophobic Interactions of Con A

FIG. 2. Chromatographic assays for the binding of a radiolabeled ligand to a protein using columns of Bio-Gel P-60 (1.2 x 24 cm) equilibrated with 0.01 M Tris, pH 7. a, Con A (10 mg/ml) plus [3H]tryptophan (2 x 10^{-5} M); b, [3H]tryptophan (2 x 10^{-5} M); c, bovine serum albumin (6 mg/ml) plus [3H]tryptophan (2 x 10^{-5} M); d, Con A (13 mg/ml) plus Me[14C]Glc (4 x 10^{-4} M); e, Con A (13 mg/ml) plus [14C]galactose (3 x 10^{-5} M); f, Con A (16 mg/ml) plus [3H]colchicine (8 x 10^{-4} M). In each experiment, a 100-μl solution was fractionated on the respective columns and fractions of 0.7 ml were collected. Aliquots of 200 μl were taken from each fraction, diluted with 5 ml of Aquasol, and subjected to scintillation counting.

Interaction of Con A with Indoleacetic Acid - Because of the obvious structural similarity between tryptophan and the auxin, β-indoleacetic acid, we have investigated the binding of this plant growth hormone to Con A and other lectins. Using the chromatographic assay on Bio-Gel P-60, we found that β-indole-[14C]acetic acid was bound to Con A, both in the presence and absence of the saccharide, MeMan (Fig. 3, a to c). Conversely, the presence of β-indoleacetic acid did not prevent the binding of Me[14C]Glc by Con A (Fig. 3d).

The stoichiometry and affinity constant for the Con A-auxin interaction were determined by equilibrium sedimentation and dialysis experiments. First, the molecular weight of Con A in 0.01 M Tris, pH 7, was found to be 104,000. An identical value was obtained in the presence of MeMan (0.1 M), β-indoleacetic acid (10^{-4} M), or both MeMan and β-indoleacetic acid. Because of the high concentration of protein and the long time required, the equilibrium dialysis experiments were performed in the Tris buffer containing MeMan (0.1 M), which inhibits the precipitation of Con A solutions (14). The results (Fig. 4) were consistent with the binding of 1 β-indoleacetic acid molecule by each subunit of Con A with an association constant of about 7 x 10^{6} M^{-1}. The curvature of the Scatchard plot at high v values suggests that additional binding sites of lower affinity may also be present.

Using equilibrium dialysis, we also investigated the binding of β-indole-[14C]acetic acid to Con A in the presence of a number of other ligands, particularly certain nonpolar compounds that have been shown to bind in a hydrophobic cavity located in the crystallographic structure of Con A. In agreement with the results of the chromatographic assay (Fig. 3, a and c), we found that the presence of MeMan had no effect on the binding of β-indole-[14C]acetic acid to Con A (Table I). In contrast, equimolar concentrations of nonpolar compounds such as tryptophan, benzoic acid, o-iodobenzoic acid, and o-iodophenol all reduced the amount of β-indole-[14C]acetic acid bound to Con A. In repeated experiments, slight precipitation was observed in the dialysis cell containing o-iodophenol and the extent of inhibition of β-indole-[14C]acetic acid binding shown for this compound may therefore have been partly due to protein denaturation. Nevertheless, the results obtained for the other hydrophobic compounds clearly indicate inhibition of β-indole-[14C]acetic acid binding to Con A.

In a number of preliminary experiments, we also found similar binding interactions between other lectins and li-
Hydrophobic Interactions of Con A

Glands such as β-indoleacetic acid and tryptophan. Succinyl-Con A, Favin, phytohemagglutinin, and the lectin from the green pea bound tryptophan and β-indoleacetic acid. In contrast, lysozyme, which also binds carbohydrate ligands, did not bind these hydrophobic molecules.

**Hydrophobic Interactions between Con A and Lipid Vesicles** - It has been reported previously that Con A interacts with lipid vesicles and that this interaction results in the aggregation, fusion, and precipitation of the protein-lipid mixture (7). We have confirmed this result and have found that when a clear solution of sonicated DPPC vesicles (2% w/v) was mixed with a clear solution of Con A (2.5 mg/ml), a thick white precipitate developed (Table II). In addition, we have observed similar precipitations of protein-lipid mixtures using other lectins such as phytohemagglutinin and Favin. In contrast, little or no precipitation of the DPPC vesicles occurred in the presence of wheat germ agglutinin, bovine serum albumin, or β-lactoglobulin.

Preliminary experiments have revealed that high concentrations (1 mM) of tryptophan and benzoic acid retarded the precipitation of DPPC vesicles by Con A. In the presence of tryptophan, for example, precipitation developed in mixtures of DPPC and Con A only after hours. This should be contrasted with precipitation which was observed within 10 min in the absence of any inhibitor. These results suggest that the DPPC may also interact with Con A via the hydrophobic binding site.

**Effect of Hydrophobic Interactions on Mitogenic Activity of Con A** - In order to study the role of the hydrophobic binding

### Table I

**Effect of various hydrophobic ligands on binding of β-indole[14C]acetic acid to Con A**

The concentrations of Con A and β-indole[14C]acetic acid were 12.6 mg/ml and 1 x 10⁻⁴ M, respectively. Experiments were performed in 0.01 M Tris, pH 7.0 at 4°C.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Concentration</th>
<th>β-indole[14C]acetic acid bound</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>1960</td>
<td>0</td>
</tr>
<tr>
<td>α-Methyl-β-mannoside</td>
<td>10⁻¹</td>
<td>2080</td>
<td>0</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>10⁻⁴</td>
<td>1723</td>
<td>12</td>
</tr>
<tr>
<td>α-Iodophenol</td>
<td>10⁻⁴</td>
<td>394</td>
<td>80</td>
</tr>
<tr>
<td>α-Iodobenzoic acid</td>
<td>10⁻⁴</td>
<td>1225</td>
<td>35</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>10⁻⁴</td>
<td>1270</td>
<td>35</td>
</tr>
</tbody>
</table>

*Values reported are averages of duplicate determinations.

### Table II

**Precipitation of DPPC vesicles by Con A and various proteins**

The concentration of DPPC was 2% (w/v). The concentration of various proteins were 2.5 mg/ml except for Favin which was ~1 mg/ml. Experiments were performed at room temperature in pH 7.6 buffer containing 150 mM NaCl, 5 mM KCl, 20 mM Heps, and 4 mM EDTA. Precipitation was scored qualitatively; -, no precipitation; +, slight cloudiness; +, increasing amounts of precipitation.

<table>
<thead>
<tr>
<th>Protein sample</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Con A</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Phytohemagglutinin</td>
<td>±</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Favin</td>
<td>±</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Wheat germ agglutinin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Con A (no DPPC)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table III

**Effect of tetracanoyl phorbol acetate (TPA) on stimulation of lymphocytes by Con A**

Mitogenic response

<table>
<thead>
<tr>
<th>[Lectin]</th>
<th>No TPA</th>
<th>10⁻⁴ M TPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>240</td>
<td>1650</td>
</tr>
<tr>
<td>5</td>
<td>9100</td>
<td>4300</td>
</tr>
<tr>
<td>25</td>
<td>5470</td>
<td>3960</td>
</tr>
<tr>
<td>50</td>
<td>3050</td>
<td>1190</td>
</tr>
<tr>
<td>100</td>
<td>1200</td>
<td>620</td>
</tr>
</tbody>
</table>

* Mitogenic stimulation is expressed in terms of the [14C]thymidine incorporated.

1 Dimethyl sulfoxide, the solvent used for TPA, was added to control cultures containing only lectin.

2 [3H]TPA (1.42 x 10⁻⁴ cpm/mmol) in MeSO was used in those experiments. The presence of the 3H-labeled mitogen did not interfere with the assay of [14C]thymidine incorporation.

**Fig. 5.** Chromatographic assays from the binding of [125I]Con A and [3H]TPA using columns of Sepharose 4B (1.5 x 30 cm) equilibrated with 0.15 M NaCl, 5 mM KCl, 0.02 M Heps, pH 7.6. a, [3H]TPA (10⁻⁴ M); b, [125I]Con A (800 µg/ml); c, [3H]TPA (10⁻⁴ M) plus [125I]Con A (800 µg/ml). In each experiment, a 240-µl solution was fractionated on the respective columns and fractions of 1 ml were collected. Aliquots of 200 µl were taken from each fraction, diluted with 5 ml of Aquasol, and subjected to γ and scintillation counting. ---, radioactivity due to [125I]Con A plotted on the scale indicated on the left hand ordinate; O--O, radioactivity due to [3H]TPA plotted on the scale indicated on the right hand ordinate.
Hydrophobic Interactions of Con A

site of Con A in the mitogenic activity of the lectin, we have tested the effects of adding hydrophobic molecules to lymphocyte cultures stimulated with Con A. Over the range of concentrations tested (10⁻⁹ to 10⁻⁷ M), neither tropothen nor β-indoleacetic acid had any effect on the stimulation of lymphocytes by Con A.

We have reported previously, however, that the addition of the phorbol ester, TPA, to human lymphocytes stimulated by Con A shifted the dose-response curve of the lectin (25). TPA, when used alone, was mitogenic for lymphocytes with an optimal concentration of about 10⁻⁶ M. The addition of a suboptimal dose of TPA (10⁻⁷ M) to cultures containing suboptimal doses of Con A greatly enhanced the response of the cells whereas an identical addition of the phorbol ester to cultures containing optimally mitogenic doses of the lectin decreased the cellular response (Table III).

In the light of these results and the fact that the tetradecanoyl moeity of TPA is hydrophobic, it was of interest to test for interactions between Con A and TPA. Chromatography of ³H-labeled TPA on a column of Sepharose 4B in Heps buffer, pH 7.6, yielded two components (Fig. 5c). The component emerging at the void volume (Component A, Fig. 5c) probably represents micelles formed by the phorbol ester itself. Analysis of the material in this component by thin layer chromatography showed that the bulk of the radioactivity migrated with an R, value identical with that of unlabeled TPA.

Chromatography of ¹²⁵I-labeled Con A showed only one component, eluting at a position corresponding to slightly less than the total volume of the column (Fig. 5f). Finally, mixing of ¹²⁵I-Con A and ³HITPA followed by chromatography resulted in the appearance of ¹²⁵I radioactivity in the void volume (Component A, Fig. 5e). The maximum value of the ¹²⁵I counts was found in the same fraction as the maximum value of the ³H counts. In addition, the profile of ³H counts showed a shoulder (Component B, Fig. 5e) that was not observed when ³HITPA was chromatographed alone (Fig. 5c).

This may represent the radioactivity due to ³HITPA bound to Con A and eluted at a position ahead of unbound TPA. Alternatively, this shoulder in the ³H radioactivity profile may be merely an artifact due to ¹²⁵I present in the Con A that is eluted at this position. In any case, the results suggest that, like DPPC vesicles, the micellar form of TPA interacts with the hydrophobic binding site on Con A.

DISCUSSION

Several lines of evidence have indicated that Con A binds to various structures via hydrophobic interactions independent of the saccharide-binding activity normally associated with the lectin. These observations have included the binding of Con A to lipid vesicles devoid of any carbohydrates (7, 8), the binding of interferon to Con A/Sepharose and dissociation of the complex using ethylene glycol (9), as well as the binding of iodine-labeled phenyl compounds to crystalline Con A (4, 10, 11). The present study confirms these previous qualitative results and conclusions and provides new information on other ligands, on binding constants, and on the relationship of the binding site to the hydrophobic cavity of Con A.

Using several independent techniques, we have quantitatively characterized the solution binding of Con A with a hydrophobic ligand, β-indoleacetic acid, that is not associated with a carbohydrate moiety. The data on the binding of β-indoleacetic acid (Fig. 4) are consistent with the presence of a single weak binding site per subunit of protein, although the possibility of other nonspecific sites cannot be excluded. In addition, competition experiments using various hydrophobic ligands such as o-iodobenzoic acid (Table I) strongly suggest that the binding site is located (Fig. 6) in the same hydrophobic cavity in which the iodobenzoic acid has been located by x-ray crystallography (10, 11). Recent evidence on the location of the saccharide-binding site of Con A (26, 27) suggest that it is at the top of the subunit, 20 Å away from the nonpolar cavity that binds the hydrophobic ligands (Fig. 6). In agreement with these observations, we have found that the binding of saccharide and hydrophobic ligands are neither competitive nor interactive: the presence of MeMan does not inhibit β-indole[¹⁴C]acetic acid binding and conversely the presence of β-indoleacetic acid does not prevent Mel[¹⁴C]Glc binding (Fig. 3, c and d).

We have previously reported other studies of the solution properties of the nonpolar binding site of Con A (10, 28). In one study (28), we found that the fluorescent probe 2-p-toluidinyl-naphthale-6-sulfonate bound to Con A with a relatively high affinity constant of 2 x 10⁴ M⁻¹ but the stoichiometry of dye binding (one site per tetramer) was not consistent with binding at the hydrophobic site in each subunit. In another study (10), we failed to detect appreciable binding of either [¹⁴C]phenol or of [¹⁴C]-labeled o-iodophenyl-β-D-glucose that is not accounted for by saccharide binding. The fact that we are now able to detect this hydrophobic binding property of Con A in solution may be due to a number of factors: (a) the presence in tropothen and β-indoleacetic acid of a larger nonpolar moiety than the simple phenyl group in phenol and o-iodophenyl-β-D-glucose; (b) the higher specific activities of the radioactive ligands; and (c) the higher concentrations of both protein and ligands used in the dialysis experiments.

Although the significance of the β-indoleacetic acid/Con A binding in the physiology of the seed is not known, the present findings do suggest several lines of speculation concerning the function of lectins. β-Indoleacetic acid is a well known auxin, a group of compounds that function as plant growth hormones (29). Con A may bind to β-indoleacetic acid and serve as a storage or transport protein for the hormone. Alternatively, inasmuch as β-indoleacetic acid is known to be active as a number of structurally similar derivatives, it is possible that Con A may play a role in its conversion to these derivatives.

No such enzymatic activity has been reported, however.

A number of lines of evidence have demonstrated that Con A and other lectins may function at the root hair wall by binding symbiotic rhizobia to the root hair (30). Although the prevailing idea is that lectins ligate the bacteria to the root...
hair through cross-reactive antigens common to the host and parasite, it is also possible that Con A binds to rhizobia and root hairs via structurally different components, one involving a carbohydrate moiety and the other a hydrophobic group. Thus, the hydrophobic site on Con A may serve functions other than hormone binding. In this connection, it should be noted that the measured affinity constant of $7 \times 10^{-8}$ m$^{-1}$ for the binding of $\beta$-indoleacetic acid to Con A indicates very weak interactions. This should be compared to binding constants of $10^{-6}$ m$^{-1}$ (29) for other macromolecular receptors for $\beta$-indoleacetic acid. It should be stated, therefore, that we are aware of the difficulty of measuring such low affinity constants reliably and of the caution that is required in interpreting the significance of such interactions. Nevertheless, the fact that this binding between $\beta$-indoleacetic acid and Con A can be inhibited by aromatic ligands such as o-iodobenzoic acid, but not by saccharides (Table I), suggests that some discrimination does exist in the binding site for hydrophobic moieties. Moreover, the association constant for the Con A-MeGic interaction (1.6 x $10^{9}$) is also a low affinity constant. It is possible, therefore, that for both saccharide and hydrophobic binding sites on Con A, the natural ligands may bear structures considerably different from the ligands that we have used to characterize the binding sites (Fig. 6).

In extending these results and ideas obtained from studies on Con A to the function of lectins in general, it is important to investigate whether other lectins also contain hydrophobic binding sites in addition to the carbohydrate-binding sites. Two lines of evidence indicate that lectins other than Con A also possess binding sites for nonpolar molecules. First, preliminary experiments have shown that Favin, phytohemagglutinin, and the lectin from the green pea bean bound tryptophan and $\beta$-indoleacetic acid, suggesting that these lectins also have hydrophobic sites. The second line of evidence comes from examination of the coat and three-dimensional structure of the hydrophobic cavity in Con A in which $\beta$-indoleacetic acid is presumed to be bound (Fig. 6). This region is surrounded by amino acid side chains of Tyr 54, Leu 81, Leu 85, Val 89, Val 91, Phe 111, Ser 113, Val 179, Ile 181, Phe 191, Phe 212, and Ile 214 (4, 10). All of these residues except Ser 113 are hydrophobic. Recent amino acid sequence studies have shown sequence homologies between Con A, lectins from lentil, green pea, soy bean, and peanut, as well as phytohemagglutinin from the red kidney bean (31). Of particular significance was the finding that the sequence of the a subunit of both lentil and green pea lectins was strikingly homologous to residues 72 to 100 of the Con A polypeptide chain (32, 33). As we indicated above, this part of the Con A polypeptide plays an important role in forming the hydrophobic cavity. This conservation of sequences between the lectins thus raises the possibility that the nonpolar binding site may have been preserved through evolution and may serve an essential role in the function of these proteins in the plant.

Regardless of the role that the nonpolar binding site may play in the function of Con A in plant seeds, the demonstration of a hydrophobic binding site that is distinct from the carbohydrate-binding activity normally associated with the lectin has a number of implications for interpretation of experiments based on the use of Con A as an adventitious mitogen for lymphocytes. We have previously reported, for example, that the addition of the phorbol ester TPA to human lymphocytes shifted the dose-response curve of the lectin (Table III) (25). In aqueous solution, TPA forms micelles (Fig. 5c), probably due to the extremely hydrophobic nature of the tetracosanoyl moiety. Moreover, a direct interaction between Con A and TPA could be demonstrated when the labeled mitogens were co-chromatographed on the same column (Fig. 5c). Similarly, Con A can bind to DPPC (7) and other phospholipid vesicles, such as dimyristoyl lecithin, resulting in a greatly enhanced binding and mitogenic activity when tested with mouse thymocytes (34). All of these findings provide a possible explanation for the observed effect of TPA on the dose-response curve of Con A inasmuch as the presence of the phorbol ester may enhance the binding of the lectin to the cell surface through both hydrophobic as well as carbohydrate interactions. We have observed that nonpolar ligands such as tryptophan inhibit Con A-vesicle interactions as assayed by DPPC vesicle precipitation. It appears likely therefore that lipid vesicles also interact with Con A via the same hydrophobic cavity involved in tryptophan and $\beta$-indoleacetic acid binding.

We have recently shown (35) that Con A derivatives that have been made monovalent with respect to carbohydrate binding were mitogenic for lymphocytes. These experiments have been interpreted in terms of the hypothesis that multi-point attachment and receptor bridging on the cell to which the mitogen is bound may not be a stringent requirement for mitogenic stimulation. The existence of a nonpolar binding site on each subunit of Con A (Fig. 6) raises the alternative possibility, however, that cross-linking of cell surface components still takes place in the monovalent lectin derivative. After initial binding to cell surface carbohydrates, monovalent Con A may still be multivalent with respect to other hydrophobic ligands of the cell and therefore may cross-link them to induce cell activation. In addition, it is also possible that Con A univalent with respect to carbohydrate binding is first bound via hydrophobic interactions to macrophages, which in turn present the mitogen in multivalent form to the responding lymphocyte. Further development of the chemistry of Con A, in which the carbohydrate binding and the hydrophobic binding sites are independently or concurrently modified, should allow a choice to be made among the possible mechanisms underlying the biological activities of the lectin.

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REFERENCES

Hydrophobic Interactions of Con A

Binding and functional properties of concanavalin A and its derivatives. III. Interactions with indoleacetic acid and other hydrophobic ligands.

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