Binding of Nonpolar Molecules by Crystalline Concanavalin A†

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ABSTRACT: Concanavalin A contains a cavity which binds a number of relatively nonpolar molecules. Methyl *p*-hydroxybenzoate, phenyl phosphate, *o*-iodobenzoic acid, *o*-iodoaniline, dimethylmercury, and *o*-iodophenyl β-D-galactopyranoside have been shown to bind to this site by X-ray crystallographic methods. It has previously been shown (Becker *et al.* (1971), *J. Biol. Chem. 246*, 6123) that *o*-iodophenyl β-D-glucopyranoside occupies this same site in crystalline concanavalin A, and since this compound inhibits dextran precipitation, it was assumed to indicate the general carbohydrate binding site. However, *o*-iodophenyl β-D-galactopyranoside and the other compounds listed above do *not* inhibit dextran precipitation and yet we have shown that they bind to this same site. Therefore, the binding of *o*-iodophenyl β-D-glucopyranoside to this cavity in the crystal is due to the iodophenyl moiety and is *not* indicative of the carbohydrate-specific binding site.

The ability of concanavalin A (Con A[1]) and other lectins to bind a variety of carbohydrates has led to many applications for this class of proteins in structural studies of polysaccharides, glycoproteins, glycolipids, cell membranes, and various aspects of cancer research.[2] Considerably more is known about the structure and biological properties of Con A than of any other lectin. The tentative amino acid sequence of the Con A monomer has been reported (Edelman *et al.*, 1972) to contain 238 residues with a molecular weight of 26,000 and its three-dimensional structure has been determined by X-ray crystallographic analysis (Edelman *et al.*, 1972; Hardman and Ainsworth, 1972b). In the crystal, four identical subunits cluster together in a pseudo-tetrahedral arrangement to form a tetramer of about 104,000 daltons (Hardman *et al.*, 1971a,b). The dominant structural features are three regions of antiparallel β-pleated sheet which involve slightly more than 50% of the residues (Hardman and Ainsworth, 1972b), and the Mn^{2+} and Ca^{2+} double ion site.

The purpose of our investigation is to determine the three-dimensional structure of Con A while complexed with other molecules. We report here that the cavity previously thought to be the general carbohydrate binding site instead binds a variety of relatively nonpolar molecules, independently of any carbohydrate binding.

Experimental Section

Procedures for Con A purification, crystallization, and data collection have been previously described (Hardman and Ainsworth, 1972b; Hardman *et al.*, 1971a,b) except as noted. The three-dimensional difference Fourier maps were calculated to a nominal resolution of about 3 Å (see figure legends for each specific complex) with structure factor amplitudes collected for each complex minus native amplitudes and phases derived from native protein structure factors. Measured structure factor amplitudes for native protein used for the difference Fourier synthesis of each complex were obtained from crystals in the same buffer as the complex. The native protein phases used were those derived with five heavy-atom derivatives for the calculation of the 2.4-Å electron density map (Hardman and Ainsworth, 1972b). Data for each complex were collected on a single crystal until the decay due to X-irradiation reached approximately 10% of the initial measured intensities. The electron density intervals for the contours in the difference maps are on an arbitrary scale and vary somewhat among the complexes because of different resolution for each data set. The identification of all amino acid side chains referred to in this text corresponds to the tentative sequence published by Edelman *et al.* (1972).[3]

Preparation of the Crystal Complexes. Crystals of the *o*-iodobenzoic acid, phenyl phosphate, and *o*-iodoaniline complexes were prepared from protein which had been demetallized by dialysis for 16 hr *vs.* 1 M acetic acid in 1% NaCl and then against H_2O until the pH reached 4.5. $MnCl_2$ and $CaCl_2$ were added to give final concentrations of 5 mM prior to crystallization in 2 M phosphate (pH 6.0). The *o*-iodobenzoic acid and phenyl phosphate crystal complexes were prepared by soaking Con A crystals in solutions which were 0.1 M (also 2 M in phosphate) for 2 weeks. *o*-Iodoaniline is much less soluble and this derivative was therefore formed by soaking crystals in a saturated solution (about 1 mM) in 2 M phosphate for 1 month. Con A for the MeHObenzoate complex was from Miles-Yeda (twice crystallized), and crystals for diffraction were grown in microdiffusion tubes, starting with the protein in saturated NaCl and dialyzing against 0.1 M Tris–nitrate buffer containing 1 mM Mn^{2+} and 2 mM Ca^{2+}. The crystals were harvested into the same buffer plus 0.1 M phosphate which was saturated with MeHObenzoate, about 5 mM, and soaked for 6 days.

Crystals of the βIphGal*p* complex were prepared by soaking crystals (grown in 0.1 M Tris–nitrate, pH 6.5) in the same buffer

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[1] Abbreviations used are: Con A, concanavalin A; MeHObenzoate, methyl *p*-hydroxybenzoate; αMeGlc*p*, methyl α-D-glucopyranoside: αMeMan*p*, methyl α-D-mannopyranoside; βIphGlc*p*, *o*-iodophenyl β-D-glucopyranoside; βIphGal*p*, *o*-iodophenyl β-D-galactopyranoside; Me_2Hg, dimethylmercury.

[2] For a short review of the biological effects of Con A and other lectins and an appropriate list of references, see Sharon and Lis (1972).

[3] We currently have 237 amino acids built into our model. According to the tentative sequence (Edelman *et al.*, 1972), residues 71 and 72 are Asx. This region is at a corner (see Figure 7) and the model fits the contours better by deleting one of these residues. In numbering the residues in the figures for this article, residue 72 has been skipped so that the numbered amino acids correspond to those published by Edelman *et al.* (1972).

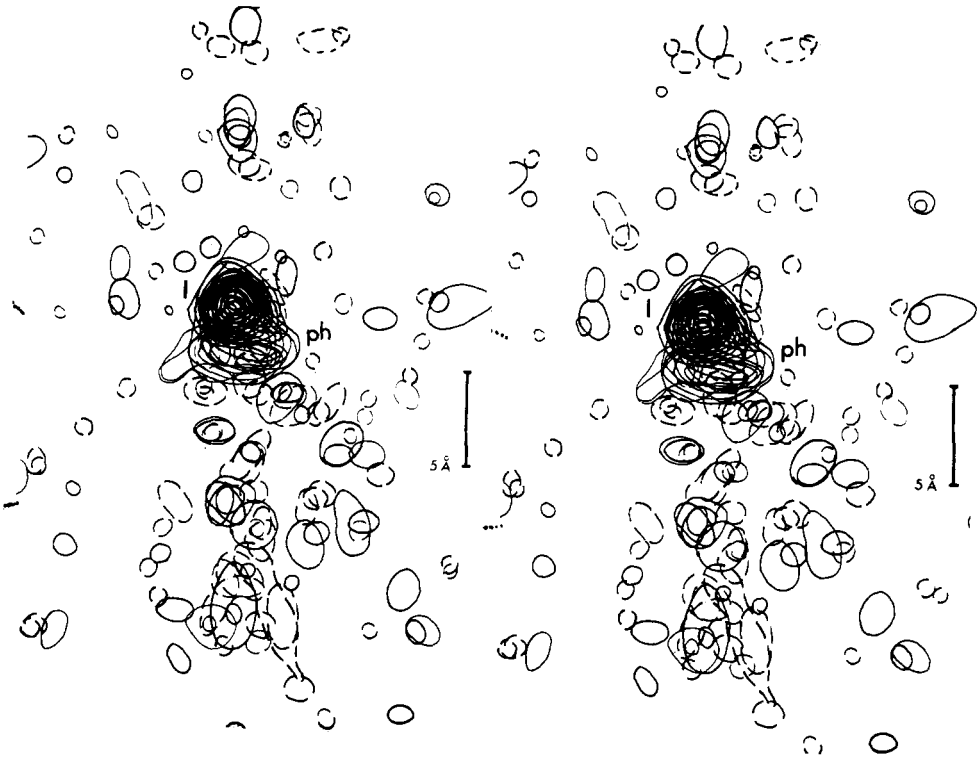

FIGURE 1: Stereogram of the difference electron density map of the cavity region for the βIphGal*p* complex at 2.48-Å resolution. The "I" labels the contours of the iodine position and "ph," the phenyl ring. The orientation of the crystallographic axes are the same in all figures (see Figure 7). All difference maps (Figures 1–5) contain 7 serial x sections from $x = 7.4$–14.7 Å and have the same scale.

saturated with the sugar (approximately 5 mM) for 11 days. The synthesis of βIphGal*p* was carried out by Dr. C. F. Brewer and is to be reported elsewhere.

The Me_2Hg derivative was prepared in a ventilated hood by adding this compound (liquid, very volatile, and toxic) directly into the quartz capillary containing the crystal for data collection. A 5-mm length of polyethylene tubing (0.6-mm i.d.) was filled with Me_2Hg and placed inside the quartz capillary, which was then sealed with an oxygen torch.

Inhibition of Dextran Precipitation. The ability of various molecules to react with the carbohydrate-specific site of Con A was measured by the inhibition of Con A–dextran precipitation. The method utilized Blue Dextran 2000 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J. 08854) and was similar to that described by Sly (1972). All solutions except the protein stock were made in buffer containing 0.01 M Tris, 1 mM $MnCl_2$, 2 mM $CaCl_2$, and 0.2 M NaCl (pH 7.0), and the reactions were carried out at room temperature (22°). The protein stock contained 2.3 mg/ml of Con A and 1.2 M NaCl. To 100 μl of protein stock, 300 μl of buffer containing the appropriate amount of compound to be tested was added and allowed to stand for more than 24 hr. Blue Dextran 2000 (100 μl, 2.5 mg/ml) was added and again the solutions stood for over 24 hr. The solutions were then centrifuged in a clinical centrifuge and the absorbances were read in a Zeiss PMQ II spectrophotometer at 630 nm with 0.5-ml cuvettes. Controls used for no inhibition contained buffer, protein, and Blue Dextran, and for 100% inhibition, buffer and Blue Dextran. Absorbancies ranged from 0.310 to 0.450, respectively. All samples were tested in duplicate.

Results

Difference Electron Density Maps. The mean values for the unit cell dimensions of nine native crystals (space group *I*222) were 63.15 ± 0.024,[4] 86.91 ± 0.053, and 89.25 ± 0.042 Å for a, b, and c, respectively (Hardman and Ainsworth, 1972b). The maximum deviations from these values for all six complexes reported here were 0.14, 0.22, and 0.07 Å, respectively. These complexes all show peaks with maxima at 11.85 ± 0.62, 13.5 ± 0.85 and −0.62 ± 0.85 Å, respectively (Figures 1–5). MeHObenzoate, o-iodoaniline, Me_2Hg, and βIphGal*p* each show only a single peak in the asymmetric unit, whereas o-iodobenzoic acid and phenyl phosphate show additional peaks. Table I lists the coordinates of the electron density peaks for these complexes with approximate occupancies.

Figure 1 shows the electron density differences produced by the binding of βIphGal*p* by Con A crystals. The highest contours clearly define the iodine position to which are attached the contours of the phenyl ring. A number of small positive and negative contours appear in the region below the iodophenyl ring which result from a small shift in the section of polypeptide chain from residues 182 to 184. No electron density contours are present which could represent the position of this carbohydrate moiety, which likewise was reported for the βIphGlc*p* complex (Edelman *et al.*, 1972). Since the position of the iodine is ortho, the general position of the galactopyranose group must be to either the left or right (Figures 1 and 6) of the iodine peak. Placing a model of βIphGal*p* in the cavity of the native Con A model indicates a preference for the lower left region, which is the center of the entire cavity region. The alternate choice would necessitate movements of residues which are not indicated by the difference electron density map. If βIphGlc*p* or βIphGal*p* were binding in this cavity by specific interactions between the protein and the pyranose group, a difference electron density

[4] Values after ± are standard deviations.

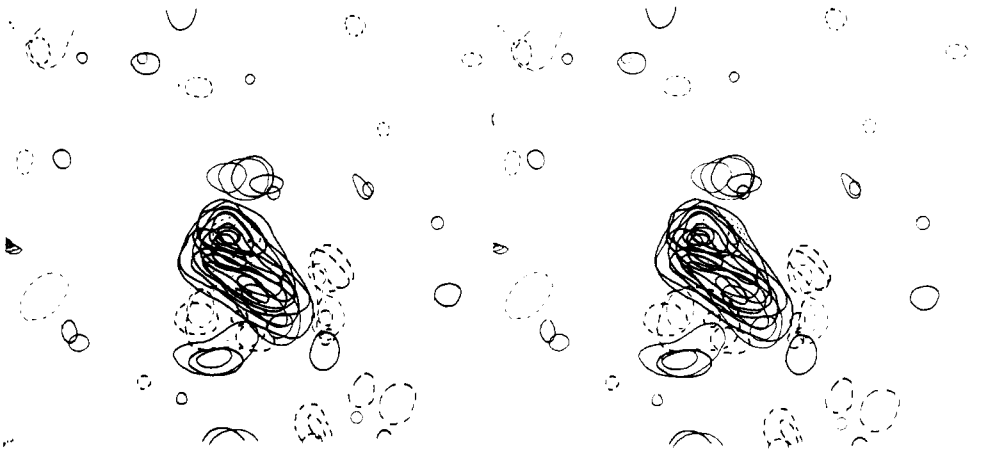

FIGURE 2: Stereogram of the difference electron density map for the MeHObenzoate complex at 2.7-Å resolution.

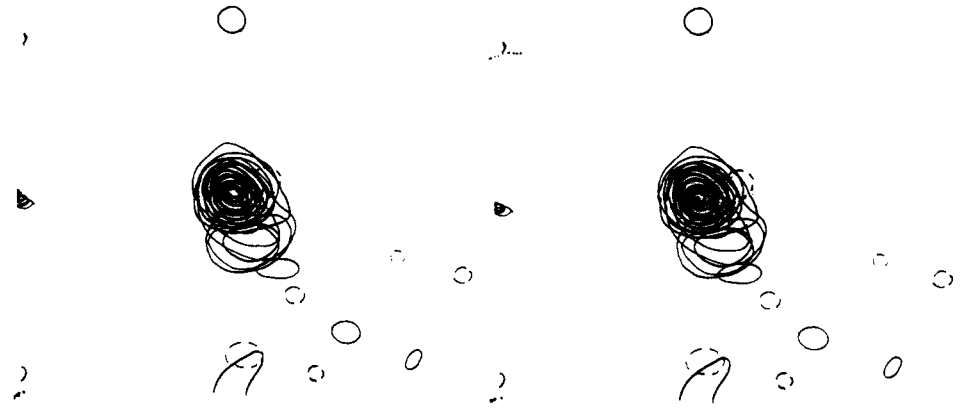

FIGURE 3: Stereogram of the difference electron density map for the *o*-iodoaniline complex at 3.0-Å resolution.

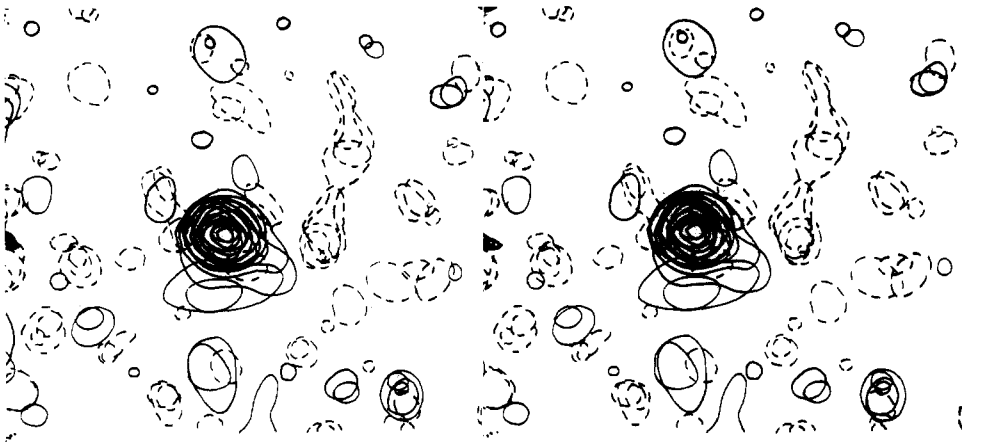

FIGURE 4: Stereogram of the difference electron density map for the *o*-iodobenzoic acid complex at 3.25-Å resolution.

map at a resolution of 3 Å or greater would be expected to show the sugar moiety.

The MeHObenzoate difference map is shown in Figure 2. The orientation is very similar to the iodophenyl group in Figure 1, with the plane of the molecule again clearly indicated. Positive and negative peaks clearly indicate shifts in positions of the side chains of Phe-192 and Ile-182.

The iodine positions for *o*-iodoaniline (Figure 3) and *o*-iodobenzoic acid (Figure 4) are identical with the iodine position for βIphGal*p* (Figure 1). Single and double contours suggest the phenyl ring positions which are consistent with βIphGal*p* and MeHObenzoate. The *o*-iodoaniline difference map shows very few contours other than the main peak and those associated with Phe-192 and Ile-182. In contrast, *o*-iodobenzoic acid produced a number of other peaks (Table I) including those near His-128 and Met-130 (site 2 and 3) where most heavy-atom derivatives bound (Hardman and Ainsworth, 1972b). The minor sites for *o*-iodobenzoic acid (4–8 of Table I) are all near positively charged side chains, Lys-39, Arg-91, Lys-201, His-206, and His-122, respectively. Binding to these sites appears then to be due to the anionic carboxyl group rather than a large number of van der Waals

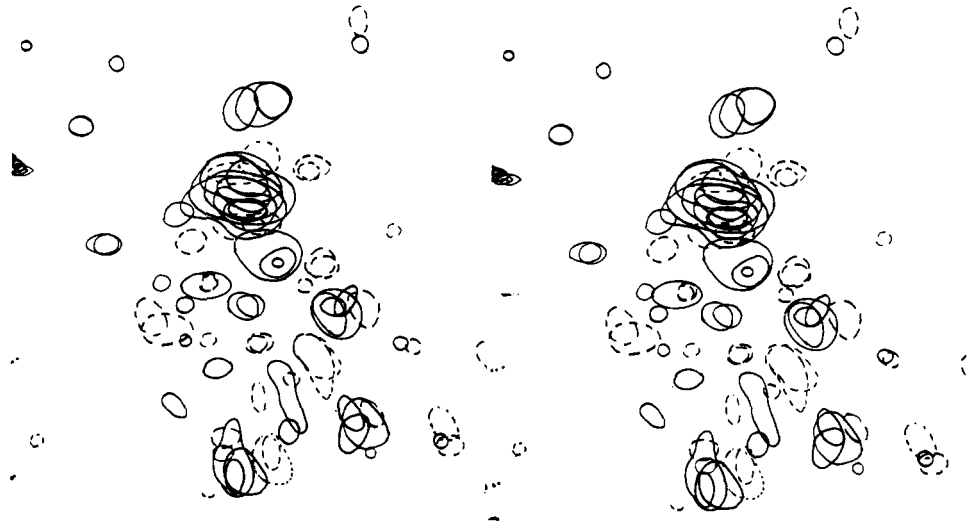

FIGURE 5: Stereogram of the difference electron density map for the phenyl phosphate complex at 2.84-Å resolution.

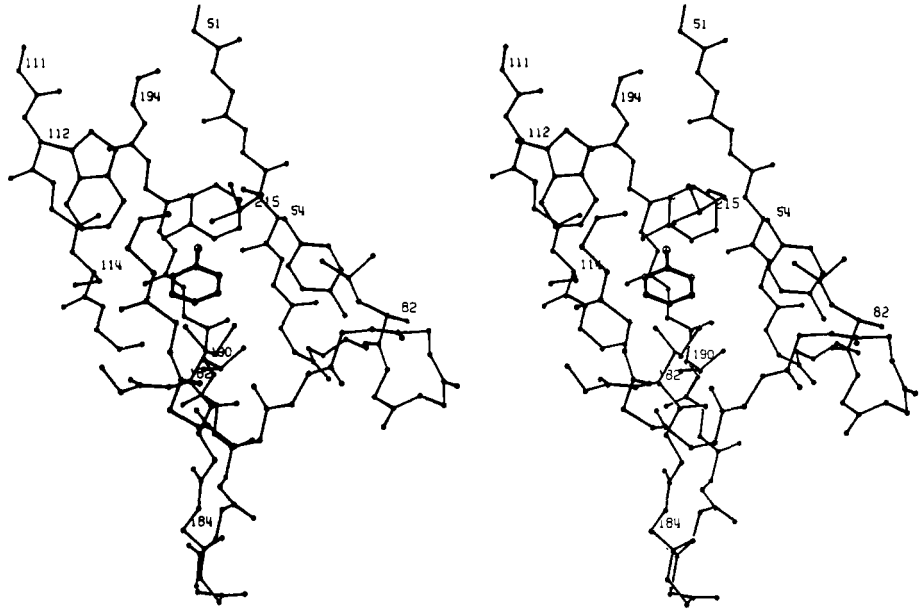

FIGURE 6: Stereogram of the nonpolar binding cavity region of the Con A subunit. The amino acid numbers are positioned near the α carbon of the corresponding residues and the atomic coordinates were taken from the Kendrew model of the native protein at 2.4-Å resolution (Hardman and Ainsworth 1972b). The iodophenyl ring (darker bonds) for the βIphGal$p$ complex was positioned by the difference map shown in Figure 1. Figures 6 and 7 were drawn by the ORTEP computer program written by Johnson (1965).

contacts, which are present in the nonpolar binding cavity. Phenyl phosphate (Figure 5) also binds in this cavity and to a much smaller extent to positively charged groups of residues His-122, Arg-91, and Lys-39 (Table I) for sites 2, 3, and 4, respectively. The Me_2Hg derivative (Table I) was collected to 6-Å resolution only and appeared as a spherical electron density (not shown) at a position corresponding to the phenyl ring positions of the other compounds.[5]

Binding Cavity. The framework for the region of the subunit near the nonpolar binding site (Figure 6) is provided by strands of polypeptide chains of 2 β-structure sheets (Figure 7). Portions of three strands of the lower sheet form the region behind the site (Figure 6). The cavity is surrounded by many nonpolar side chains, Tyr-54, Leu-82 and -86, Val-92 and -180, Ile-182 and -215, Trp-112, and Phe-192. The dimensions of the cavity are approximately 15-Å deep with a cross section of about 9 × 6 Å (from atom center to center). The phenyl ring position of these complexes lies about 10 Å from the surface of the subunit (Figure 6, lower right). The unit cell dimensions remain very constant and only a few residues are displaced slightly and the complexes are extremely isomorphous with the native structure. Leu-182 through Glu-184 move about 2 Å (downward in Figure 6) and Phe-192 moves upward by rotation about the C^{α}–C^{β} bond approximately 50°. In addition, the center of this cavity is more than 20 Å from the Mn^{2+} (Figure 7).

Inhibition of Dextran Precipitation. The ability of this assortment of compounds to bind with the specific carbohydrate binding site of Con A in solution was tested by measuring their inhibitory effect on Con A–Blue Dextran precipita-

[5] This isomorphous derivative would have produced an excellent *single-site* heavy-atom derivative for the structure determination of the native protein but was discovered after the 2.4-Å map had been produced.

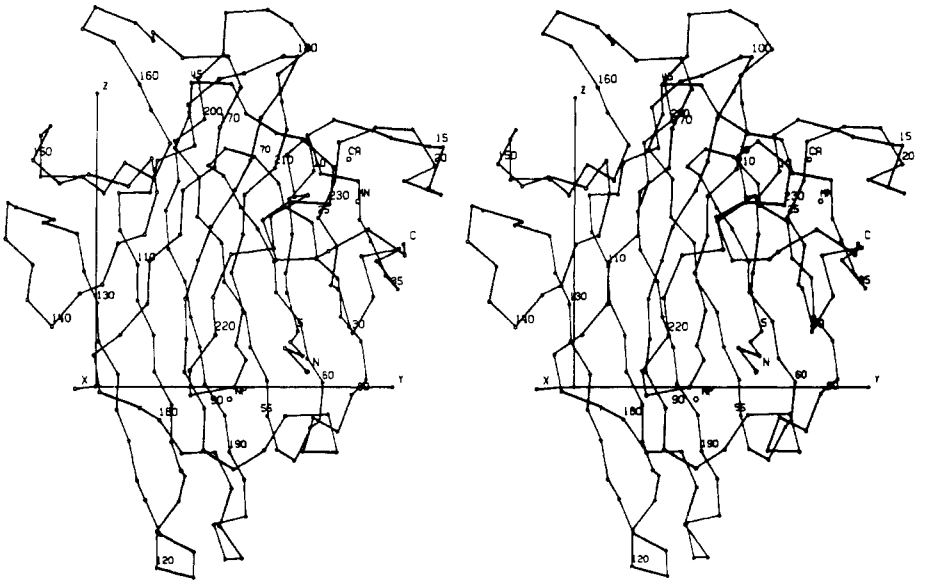

FIGURE 7: Stereogram of the α-carbon positions for the Con A monomer. The positions for the nonpolar binding cavity, Mn^{2+}, Ca^{2+}, amino, and carboxyl termini are labeled NP, MN, CA, N, and C, respectively.

tion. These results are summarized in Table II. Concentrations of αMeMan*p* from 10 to 120 μM inhibited the precipitation from 7 to 96%. Concentrations of 5 mM βIphGlc*p* produced almost complete inhibition while the same concentrations of βIphGal*p* produced *no* inhibition. Concentrations from 5 to 20 mM for MeHObenzoate, *o*-iodoaniline, *o*-iodobenzoic acid, and phenyl phosphate had no inhibitory effect; however, 100 mM *o*-iodobenzoic acid, phenyl phosphate, and *myo*-inositol showed small inhibitory effects. At these higher concentrations, the inhibitory effects of *o*-iodobenzoic acid and phenyl phosphate are likely due to binding at a number of nonspecific sites throughout the molecule (Figures 3 and 4, and Table I) rather than direct interaction with the carbohydrate binding site.

TABLE I: Binding Sites.

Compound	Site	Ångstroms			Approx Occupancy (%)
		x	*y*	*z*	
MeHObenzoate	1	12.0	12.7	−0.2	60
o-Iodoaniline	1	11.7	12.6	0.4	70
Me_2Hg	1	11.6	14.5	−1.5	85
o-Iodobenzoic acid	1	12.5	12.8	−0.4	64
	2	2.9	2.7	−2.8	65
	3	2.7	0.2	5.2	42
	4	30.4	14.2	17.1	34
	5	27.6	27.3	17.5	33
	6	24.9	4.5	−4.1	31
	7	17.7	23.4	30.3	29
	8	15.0	8.8	32.6	29
Phenyl phosphate	1	10.7	13.4	−2.0	60
	2	18.1	35.5	25.5	36
	3	0	0	17.1	35
	4	16.9	28.2	39.1	34
βIphGal*p*	1	12.6	12.3	0	100
βIphGlc*p*[a]	1	12.3	12.6	0	

[a] From Becker *et al.* (1971), translated to fit our choice of axes and origin.

Discussion

Binding of Nonpolar Molecules. The location of MeHObenzoate, *o*-iodoaniline, *o*-iodobenzoic acid, phenyl phosphate, and Me_2Hg in this cavity in crystalline Con A clearly exhibits the ability of this site to bind a wide variety of relatively nonpolar molecules. The only common feature of the first 4 is the benzene ring. The position and orientation of this ring in the Con A complex are essentially the same for these molecules and the binding appears to involve π stacking with the aromatic side chain of Phe-192 (Figure 6). Me_2Hg provides the exception here, having no aromatic ring. Other Hg compounds were used as heavy-atom derivatives in the structural determination of the native protein, $Hg(AcO)_2$, *o*-ClHgphenol, MeHgCl (Hardman and Ainsworth, 1972b), and phenylmercuric acetate (Hardman *et al.*, 1971a). These compounds, however, bind to 3–6 sites and possess a group replaceable with a nucleophile from the protein. The Me_2Hg

TABLE II: Inhibition of Blue Dextran Precipitation.

Compound	Concn (mM)	% Inhibn
αMeMan*p*	0.010	7
	0.034	50
	0.070	93
	0.120	96
βIphGlc*p*	5	99
βIphGal*p*	5	0
MeHObenzoate	5	1
o-Iodoaniline	5	0
o-Iodobenzoic acid	10	0
	100	19
Phenyl phosphate	20	0
	100	7
myo-Inositol	100	5

appears to be unreactive to protein nucleophiles and binds in this cavity solely through many van der Waals contacts with the side chains which surround it. Two of these compounds have ionizable groups which are charged near neutral pH values, *o*-iodobenzoic acid, $pK_a = 2.86$, and phenyl phosphate, $pK_{a1} = 0.80$, and $pK_{a2} = 5.63$, whereas the NH_2 of the *o*-iodoaniline, $pK_a = 2.60$, and the hydroxyl of MeHObenzoate, $pK_a = 8.34$, are not.

The molecules with iodine all appear to bind positioned so that the iodine is the atom deepest in the cavity and presumably the phosphate, NH_2, and COO^- are positioned toward the opening. The three-dimensional shape of the MeHObenzoate contours (Figure 2) suggests the –COOMe group is positioned approximately where the iodine is located in the other derivatives, which would place the para-positioned phenolic hydroxyl group pointing toward the surface. Accommodation of these polar groups attached to the phenyl ring appears to indicate the accessibility of the interior of this cavity to solvent.

The relatively clean difference electron density maps produced by the *uncharged* molecules in comparison to those with charges may be a result of a lack of secondary binding to many sites since the lower solubility of these molecules prevented testing them at higher concentrations. The association constants for these molecules binding to Con A in solution have not been measured.

Several attempts to repeat the binding of *myo*-inositol to this cavity as has been previously reported (Hardman and Ainsworth, 1972a) have produced electron density maps devoid of any significant positive or negative peaks. To prevent the growth of molds during crystallization, small amounts of the mold inhibitor MeHObenzoate were added to some preparations of Con A, including some commercial samples. Apparently this compound had not been completely washed from the crystal used for the previous report, although the possibility cannot be eliminated that the difference peaks may have been produced by the inadvertant presence of some other molecule.

There is apparently no knowledge of any natural function for Con A in the jack bean or any fortuitous biological function in other systems that has been attributed to the binding of nonpolar molecules.[6] Functions have been generally shown to result from its ability to bind specific carbohydrates (Sharon and Lis, 1972). However, Jaffé and Palozzo (1971) reported isolating nonpolar molecules, apparently steroids, by extracting Con A preparations with nonpolar solvents. It might be speculated than Con A could function in regulation of cell division or germination through binding some relatively nonpolar molecules which serve as plant growth factors, or cytokinins.

Binding of Iodophenyl Sugars. Carbohydrates with the minimum specificity for Con A binding contain hexose residues with the D-*arabino*-pyranoside configuration at C-3, C-4, and C-5 (Goldstein *et al.*, 1965). The monosaccharide found to have the highest affinity to Con A is αMeMan*p*, followed by the 2-deoxy derivative, and αMeGlc*p* with about 3- and 4-fold less affinity, respectively (Poretz and Goldstein, 1970). Galactose, the C-4 epimer of glucose, however, does not bind (Goldstein *et al.*, 1965; Hassing and Goldstein, 1970). βIphGlc*p* was used by Becker *et al.* (1971) in an attempt to establish the location of the saccharide binding site in the crystalline state by identifying the position of the iodine atom. A single electron density peak was found per monomer for the iodine in the cavity where we have found the highest density for the iodine derivatives in Table I. However, the analog with the *galactopyranose* configuration binds to the same site (Table I and Figure 1). Inhibition of dextran precipitation (Table II) clearly shows that at 5 mM, βIphGlc*p* binds very well to the carbohydrate-specific site of Con A in solution, while βIphGal*p* does *not*. If the region of the cavity adjacent to the iodophenyl binding site in crystalline Con A were the carbohydrate-specific site, the limited size of the cavity suggests that βIphGal*p* would inhibit dextran precipitation for steric reasons.

Nuclear magnetic resonance studies of the binding of αMeGlc*p* and αMeMan*p* to Con A indicate that the distances to Mn^{2+} for the carbon atoms of these sugars are between 9 and 11 Å (Brewer *et al.*, 1973; J. Villafranca, personal communication). The center of the nonpolar cavity is approximately 22 Å from the Mn^{2+}. Therefore, the βIphGlc*p* appears to bind to the Con A subunit at *two* different sites, 1, at the nonpolar cavity through interactions with the iodophenyl group alone, and 2, at a carbohydrate-specific site primarily through interaction with the glucopyranose group. Crystals of native Con A are cracked and dissolved by concentrations of αMeMan*p* above 1 mM and difference electron density maps of crystals soaked in lower concentrations showed no significant changes (K. D. Hardman and C. F. Ainsworth, unpublished observations). βIphGlc*p* concentrations above 5 mM also dissolved native crystals whereas 5 mM βIphGal*p* had no effect. One can then speculate that the carbohydrate-specific site most likely involves a region with intermolecular contacts in the crystalline lattice. The region with the largest number of such interactions involves side chains of Asp-16, Arg-229, Tyr-12, Tyr-101, which cluster around a point about 12 Å from the Mn^{2+} ion and about 7 Å from the Ca^{2+} ion.

Acknowledgments

We thank C. F. Brewer for his discussions, for suggesting the βIphGal*p* binding experiment, and for kindly supplying the compound.

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[6] Added in Proof. It should be noted that agglutination of certain tumor cells by Con A may partially involve nonpolar sites. Noonan and Burger (1973) have recently reported that Con A binding to cells is *not* totally dependent on a carbohydrate receptor site, since at 22° αMeMan*p* releases only about 50% of the Con A bound to the membrane.

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Interactions of Saccharides with Concanavalin A. Mechanism of Binding of α- and β-Methyl D-Glucopyranoside to Concanavalin A as Determined by ^{13}C Nuclear Magnetic Resonance†

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ABSTRACT: Binding of α- and β-methyl D-glucopyranoside (uniformly labeled with 14% ^{13}C) to concanavalin A was studied by pulsed Fourier transform carbon magnetic resonance techniques. The spin-lattice relaxation times (T_1) for the carbon resonances of the two glycosides were measured in the presence and absence of the zinc and manganese derivatives of the protein. T_1 values for the ring carbons of both sugars were uniformly shortened when bound to zinc concanavalin A and selectively shortened when bound to manganese concanavalin A. The results indicate that the paramagnetic managanese ion in concanavalin A contributes to the relaxation of the carbon atoms of the bound sugars. The distance between each carbon atom of the bound sugars and the manganese ion was calculated from the paramagnetic contribution of manganese to the T_1 of the sugar carbons. These measurements establish the three-dimensional orientation of both anomers relative to the transition metal site in the protein. Both sugars appear to bind in different orientations while remaining in the C-1 chair conformation with their nonreducing ends closest to the manganese ion at a mean distance of 10 Å. The distance separating the transition metal and sugar binding site in the protein, as determined from these measurements in solution, differs from the value recently proposed on the basis of X-ray diffraction studies. The different binding orientations of α- and β-methyl D-glucopyranoside account for the difference in binding constants of the two sugars and the relative affinities of their derivatives for concanavalin A.

Concanavalin A (Con A),[1] a protein isolated from the Jack bean (*Canavalia ensiformis*) (Sumner and Howell, 1936), is a member of the lectin class of plant proteins. Interest in Con A stems from its unusual effects on animal cells (*cf.* Sharon and Lis, 1972). The protein agglutinates cells transformed by oncogenic viruses (Inbar and Sachs, 1969), inhibits growth of malignant cells in experimental animals (Shoham *et al.*, 1970), and exhibits mitogenic activity (Powell and Leon, 1970). Fibroblasts transformed by oncogenic viruses *in vitro* are restored to normal cell density by exposure to trypsin-treated Con A (Burger and Noonan, 1970).

The biological effects of Con A appear to be related to its sugar binding properties. Con A binds monosaccharides with the D-mannopyranoside configuration at the 3, 4, and 6 positions (Goldstein *et al.*, 1965b), and interacts preferentially with the α anomers of these sugars. The ability of Con A to precipitate certain polysaccharides has served as a model for antibody–antigen reactions (Goldstein *et al.*, 1965a).

Between pH 3.5 and 5.6, Con A exists as a dimer with a molecular weight of 54,000; at higher pH, tetramers form (Kalb and Lustig, 1968; McKenzie *et al.*, 1972). Each monomeric unit has one site (S_1) that binds transition metal ions and another site (S_2) that binds calcium ions. Evidence has been presented suggesting that both the S_1 and S_2 sites in the protein must be occupied for saccharide binding activity to occur (Kalb and Levitzki, 1968). Derivatives of Con A in which Zn^{2+}, Co^{2+}, or Mn^{2+} ions occupy the S_1 site possess equal abilities to bind sugars. The S_1 site has been shown by X-ray diffraction studies to be located at the base of a cavity extending to the surface of each monomer (Weinzierl and Kalb, 1971). The primary sequence and electron density map of the protein at 2-Å resolution (Edelman *et al.*, 1972) and at 2.4-Å resolution (Hardman and Ainsworth, 1972) have recently been reported.

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[1] Abbreviations used are: Con A, concanavalin A; α- and β-MeGlc, α- and β-methyl D-glucopyranoside.