

LECTINS: A CHEMICAL APPROACH TO THE CELL SURFACE

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ABSTRACT

Lectins are a group of plant proteins that bind to cells through the carbohydrate moieties of glycoproteins and glycolipids on the cell surface. These proteins are proving particularly valuable in mapping receptors and studying dynamic processes on the surfaces of animal cells. Some of the uses, chemical properties and biological activities of lectins are discussed in terms of the lectin concanavalin A (Con A), which is obtained from the jack bean. This protein is the first lectin for which the amino acid sequence and three-dimensional structure have been determined. The results of these studies have defined the over-all features of the molecule, the details of the metal-binding sites and the interactions of the subunits.

Con A is composed of identical subunits each of which contains 237 amino acid residues, one Mn^{2+} ion, one Ca^{2+} ion and one binding site for carbohydrate. Each protomer is dome-shaped, with dimensions of about $42\text{\AA} \times 40\text{\AA} \times 39\text{\AA}$. At physiological pH the predominant form of the lectin is the tetramer of these subunits, but under acidic conditions, or after chemical modification with certain reagents, dimers are formed. Binding of Con A to the cell surface is necessary for initiation of the biological activities, and binding is inhibited by simple sugars such as glucose or mannose but not by closely related stereoisomers such as galactose. Con A has been used to study a variety of normal and transformed cells. Studies of the biological effects of Con A on lymphocytes have led to hypotheses on transmembrane regulation of signals which may occur on a variety of cell types.

INTRODUCTION

Analyses of the molecular processes involved in cell differentiation, cell-cell recognition and the control of cell growth have focused attention on a number of plant proteins collectively termed lectins^{1,2}. Lectins bind to a variety of cells, presumably via the carbohydrate portions of glycoproteins and glycolipids on the cell surface. There are hundreds of lectins from various plants, and among these proteins there is a wide range of structural properties, carbohydrate specificities and biological activities. Although their function in plants is unknown, the binding of lectins to some animal cells leads to surface alterations as well as biochemical changes, within the cell. These plant proteins, therefore, represent a valuable set of macromolecular probes

for examining the type, number and distribution of cell-surface receptors, and for studying dynamic processes initiated at the cell surface.

The most practical uses of lectins derive from the fact that, as proteins, they can be readily labelled with specific reagents^{3,4}. Radioactive labels provide accurate estimates of the number of lectin molecules that can bind to various cells. Fluorescent labels allow microscopic analysis of the distribution and mobility of receptors, and electron-dense labels permit extension of these studies to the level of the electron microscope. In addition, lectins can be covalently coupled to solid supports and used to fractionate cells⁵ or to isolate cell-surface receptors⁶. In many cases the binding of lectins to cells is accompanied by cell agglutination, providing a simple, fast assay for lectin binding.

Despite the large number of known lectins and their extensive use in cell biology, few of these proteins have been purified to homogeneity, and little is known about the structures of these molecules. In this paper I shall review the work carried out by myself and my colleagues to relate various features of the structure and activity of one of the most widely used lectins, concanavalin A (Con A)†. We have examined the structure of Con A and some of its activities in detail. Because the structure of Con A provides a reference structure to which other lectins can be compared as well as a basis for correlating lectin structure with biological activity, this discussion focuses on Con A rather than attempting an exhaustive list of the various lectins and their uses. A number of reviews of the latter type^{2,4,7} have appeared recently.

PROPERTIES AND STRUCTURE OF CON A

Con A is a lectin isolated from the jack bean, *Canavalia ensiformis*⁸. It binds specifically to mono-, oligo- and polysaccharides with terminal non-reducing α -D-mannopyranosyl-, α -D-glucopyranosyl- or β -D-fructofuranosyl residues⁹. Con A can agglutinate a variety of somatic and germ-line cells^{10,11} and viruses¹². Transformed fibroblasts in culture are more readily agglutinated by Con A than the corresponding normal cells¹⁰. It has been shown that Con A is mitogenic for lymphocytes¹³. The protein also induces a number of other phenomena on a variety of cell types, including an insulin-like action on fat cells¹⁴, the inhibition of tumour cell migration¹⁵ and the inhibition of phagocytosis by polymorphonuclear leucocytes¹⁶.

At physiological pH the protein exists as a tetramer of identical subunits^{17,18} of molecular weights 25 500, as shown in *Figure 1*. The protomers are dome- or gumdrop-shaped, approximately 42 Å high, 40 Å wide and 39 Å thick^{19,20}. At the base the subunits are slightly smaller, being about 25 Å thick. Monomers are paired base-to-base by an exact twofold symmetry axis to form ellipsoidal dimers of about 84 Å by 40 Å. The dimers are in turn paired by additional twofold axes to form roughly tetrahedral tetramers.

Each subunit contains a total of 237 amino acid residues^{21,22}, and has binding sites for one Mn²⁺ ion, one Ca²⁺ ion and one saccharide²³. Unlike most lectins^{2,7}, Con A contains no covalently bound carbohydrate, and

† The work of my associates Dr Gerald M. Edelman, Dr John L. Wang, Dr George N. Reeke, Jr, Dr Joseph W. Becker, Dr Ichiro Yahara and Mr Gary Gunther is cited in the references.

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there is no evidence for the presence of any lipid, nucleic acid or other prosthetic group in the molecule. Like most lectins, it has few sulphur-containing amino acids, two methionines and no half-cystines. Jack beans are known to contain unusual amino acids such as canavanine²⁴, but there is no evidence for the presence of such amino acids in Con A.

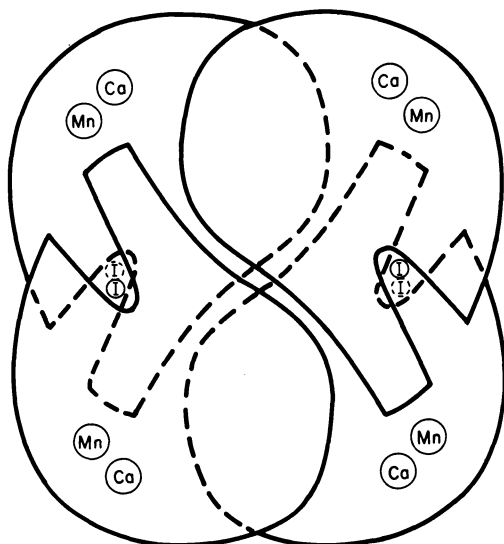


Figure 1. Schematic drawing of the Con A tetramer. I indicates the position where the iodine atom of the inhibitor β -IPG was located. Mn and Ca indicate the positions of the manganese and calcium ions, respectively

Although readily crystallized, commercially available Con A as well as fresh preparations isolated by a variety of methods consist of the intact subunit and naturally occurring fragments, A_1 and A_2 , of the intact subunit¹⁷. Procedures²⁵ have been developed for isolating the intact subunit free of fragments, and the available evidence indicates that the presence of fragments does not affect either the biological activity or the crystal structure of the molecule. The isolation of the intact subunit, however, was essential for the determination of the chemical structure of the protein^{21, 22}.

Treatment of the intact subunit of Con A with cyanogen bromide to cleave specifically at the two methionyl residues gave the expected three fragments F_1 , F_2 and F_3 ²⁶, as shown in Figure 2. Detailed analyses^{21, 22} of each of these fragments provided the amino acid sequence of the entire polypeptide chain, as shown in Figure 3. Analysis of the partial sequences of A_1 and A_2 and the products obtained after treatment of these fragments with cyanogen bromide have shown that the main point of cleavage is between residues 118 and 119^{21, 26} (Figure 2). Fragment A_1 begins at the amino terminus and extends to residue 118, while fragment A_2 accounts for the remainder of the peptide chain. It has been suggested¹⁷ that the fragments arise by proteolytic cleavage

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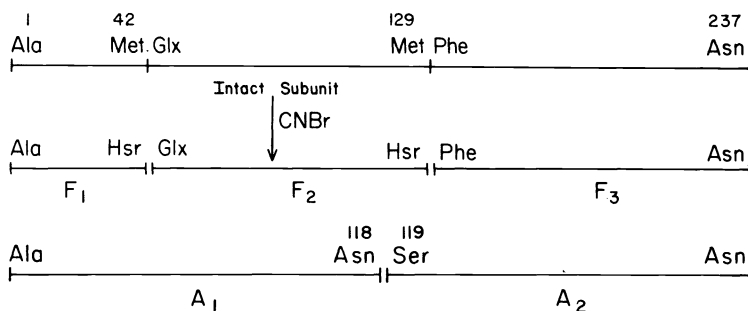


Figure 2. Size and arrangement of the CNBr fragments, F₁, F₂ and F₃, and the naturally occurring fragments, A₁ and A₂, of the intact subunit of Con A

1 Ala-Asp-Thr-Ile-Val-Ala-Val-Glu-Leu-Asp-Thr-Tyr-Pro-Asn-Thr-Asp-Ile-17

18 Gly-Asp-Pro-Ser-Tyr-Pro-His-Ile-Gly-Ile-Asp-Ile-Lys-Ser-Val-Arg-Ser-Lys-Lys-Thr-37

38 Ala-Lys-Trp-Asn-Met-Gln-Asp-Gly-Lys-Val-Gly-Thr-Ala-His-Ile-Ile-Tyr-Asn-Ser-Val-57

58 Asp-Lys-Arg-Leu-Ser-Ala-Val-Val-Ser-Tyr-Pro-Asn-Ala-Asp-Ala-Thr-Ser-Val-Ser-Tyr-77

78 Asp-Val-Asp-Leu-Asn-Asp-Val-Leu-Pro-Glu-Trp-Val-Arg-Val-Gly-Leu-Ser-Ala-Ser-Thr-97

98 Gly-Leu-Tyr-Lys-Glu-Thr-Asn-Thr-Ile-Leu-Ser-Trp-Ser-Phe-Thr-Ser-Lys-Leu-Lys-Ser-117

118 Asn-Ser-Thr-His-Gln-Thr-Asp-Ala-Leu-His-Phe-Met-Phe-Asn-Gln-Phe-Ser-Lys-Asp-Gln-137

138 Lys-Asp-Leu-Ile-Leu-Gln-Gly-Asp-Ala-Thr-Thr-Gly-Thr-Asp-Gly-Asn-Leu-Glu-Leu-Thr-157

158 Arg-Val-Ser-Ser-Asn-Gly-Ser-Pro-Glu-Gly-Ser-Ser-Val-Gly-Arg-Ala-Leu-Phe-Tyr-Ala-177

178 Pro-Val-His-Ile-Trp-Glu-Ser-Ser-Ala-Thr-Val-Ser-Ala-Phe-Glu-Ala-Thr-Phe-Ala-Phe-197

198 Leu-Ile-Lys-Ser-Pro-Asp-Ser-His-Pro-Ala-Asp-Gly-Ile-Ala-Phe-Phe-Ile-Ser-Asn-Ile-217

218 Asp-Ser-Ser-Ile-Pro-Ser-Gly-Ser-Thr-Gly-Arg-Leu-Leu-Gly-Leu-Phe-Pro-Asp-Ala-Asn-237

Figure 3. Amino acid sequence of Con A

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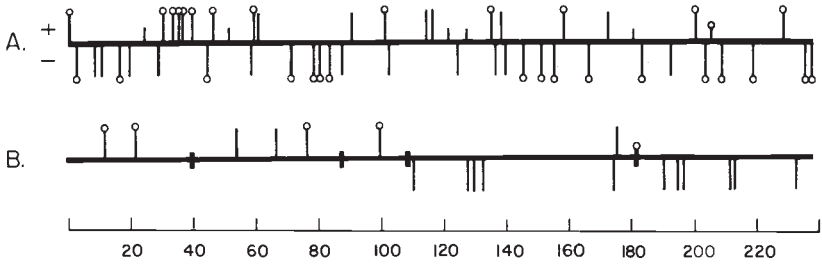


Figure 4. Distribution of (A) the charged amino acids (L) lysine, (R) arginine, (H) histidine, (E) glutamic acid and (D) aspartic acid; and (B) the aromatic amino acids (W) tryptophan, (Y) tyrosine and (F) phenylalanine in the linear structure of Con A. O denotes residues on the surface of the molecule in the three-dimensional structure

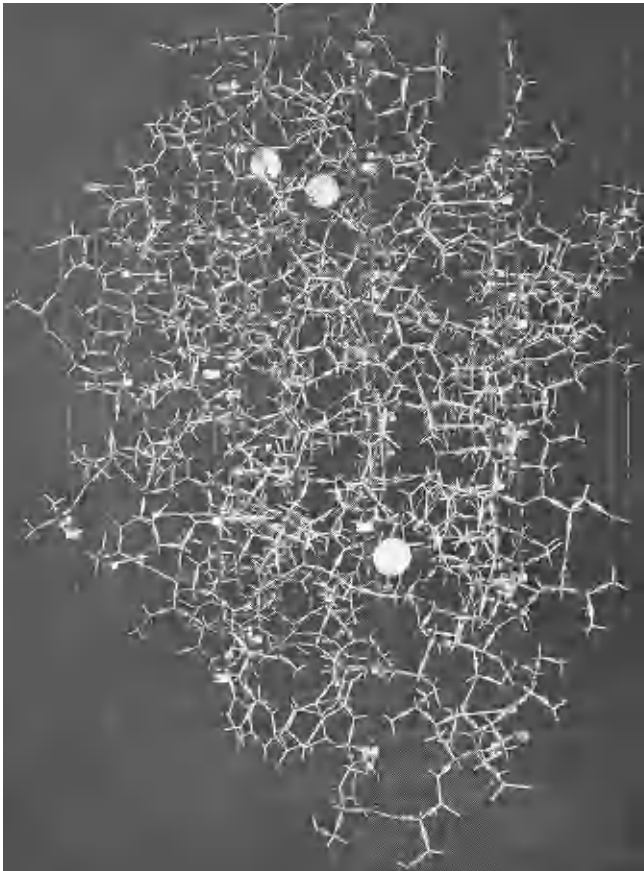


Figure 5. Kendrew model of the Con A protomer viewed towards the opening of the β -IPG binding site with the 'back' of the molecule to the right. The two spheres at the top represent the Ca^{2+} and Mn^{2+} ions, and the sphere near the bottom represents the iodine atom of β -IPG

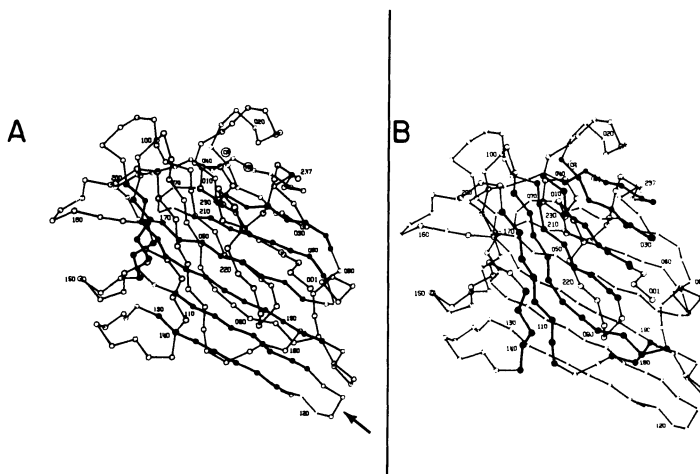


Figure 6. Front view of the Con A protomer showing the folding of the polypeptide chain with (A) the back β -pleated sheet highlighted and (B) the front β -pleated sheet highlighted. The arrow indicates the position where molecules containing fragments A_1 and A_2 are cleaved

of the lectin in the jack bean. If so, these results suggest that this cleavage is highly specific.

Detailed examination²² of the amino acid sequence shows an unusual distribution of certain amino acids in Con A (Figure 4). The distribution of charged residues (Figure 4A) is generally more dense in the NH_2 terminal half of the polypeptide chain than in the COOH portion, separating the molecule into two distinct regions at about residue 110. This division is even more striking when the distribution of aromatic amino acids is examined (Figure 4B). Six of the seven tyrosines are located in the NH_2 terminal half of the molecule, and all of the 11 phenylalanines are between residue 111 and the COOH terminus. The four tryptophanyl residues are evenly distributed. Many of these aromatic residues are located in hydrophobic regions in the three-dimensional structure of the protein^{27, 28}.

High-resolution crystallographic studies²⁷⁻³⁰ have revealed a number of details about the Con A protomer, including a number of regular structures that allow for an elegantly simple set of subunit interactions. A model of the Con A protomer at 2 Å resolution²⁹ is shown in Figure 5, and the folding of the polypeptide chain^{27, 28} is shown from a different view in Figure 6. The most striking feature of the structure is the presence of two large β -structures or pleated sheets which contain more than half the residues in the molecule. The large amount of β -structure found is consistent with earlier circular dichroism and optical rotatory dispersion studies^{31, 32}. One of the pleated sheets forms almost the entire back surface of the molecule (Figure 6A), including the rear of the β -IPG binding cavity³³, and is associated with most of the interactions involved in dimer and tetramer formation. The sheet contains 64 residues arranged in six antiparallel chains and nine residues in short connecting loops. As shown in Figure 6A, the site of natural cleavage between residues 118 and 119 is located at the end of a loop that joins the

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bottom two chains. This loop extends out into the solvent and would likely be readily accessible, particularly in the dimer, for the hypothesized enzymatic cleavage to occur. In addition, because the structure in this region is stabilized by the adjacent strands of β -structure, the cleavage would not be expected to have a significant effect on the folding of the polypeptide chain.

The second pleated sheet (*Figure 6B*) extends at an angle from the upper rear of the molecule to the left front. It contains about 57 residues arranged in seven chains, which, like those of the back β -structure, are antiparallel. This sheet divides the remainder of the molecule unequally into two randomly coiled regions.

The general distribution²⁸ of polar and nonpolar side chains in Con A is similar to that found in other proteins, with polar side chains usually on the surface and hydrophobic groups predominantly in the interior. An example of this distribution in terms of the linear structure is indicated in *Figure 4*. There are at least three salt bridges formed on the surface of the molecule between side chains of a single protomer, and the majority of the hydrophobic residues are in two hydrophobic cores on the inside of the molecule separated from each other by the front β -structure.

SUBUNIT INTERACTIONS

At physiological pH the Con A molecule exists predominantly as a tetramer of the identical subunits. Below pH 6, however, the predominant form is the dimer¹⁸. Derivatization of the protein with succinic anhydride, acetic anhydride³⁴ or maleic anhydride³⁵ produces species which retain their carbohydrate-binding activity but are dimers at physiological pH. Although these dimers bind carbohydrate, the biological activities are not identical with those of the native tetrameric protein³⁴, suggesting that the charge, valence or interactions of the subunits play an important role in these activities.

In terms of the Con A monomer, the β -structure located at the back of the molecule plays a major role in the interactions that form dimers and tetramers^{28, 29}. In the dimer the two monomers are related by a twofold axis to give the arrangement shown in *Figure 7*. Main-chain nitrogen and carbonyl oxygen atoms of Ala 125, His 127 and Met 129 in the bottom chain of the back β -structure are hydrogen-bonded to the corresponding atoms in the other monomer, forming a single large pleated sheet of 12 antiparallel chains across the entire back of the dimer, as highlighted in *Figure 7*. At least eight other hydrogen bonds and a number of non-polar interactions hold the dimer together across the area of contact.

The large pleated sheet at the back of each dimer is also important in the formation of the tetramer, as shown in *Figure 8*. In this figure only the two large back-pleated sheets in the two dimers that make up a single tetramer are shown. The sheets fit very closely, but they are slightly concave, leaving a solvent-filled cavity at the centre. Half of the side chains from each pleated sheet project into this region, where they interact to stabilize the tetramer. In this region there are a total of about 12 salt links, 36 hydrogen bonds and several hundred atoms in van der Waals contact.

Interactions between dimers include four pairs of salt links between Lys

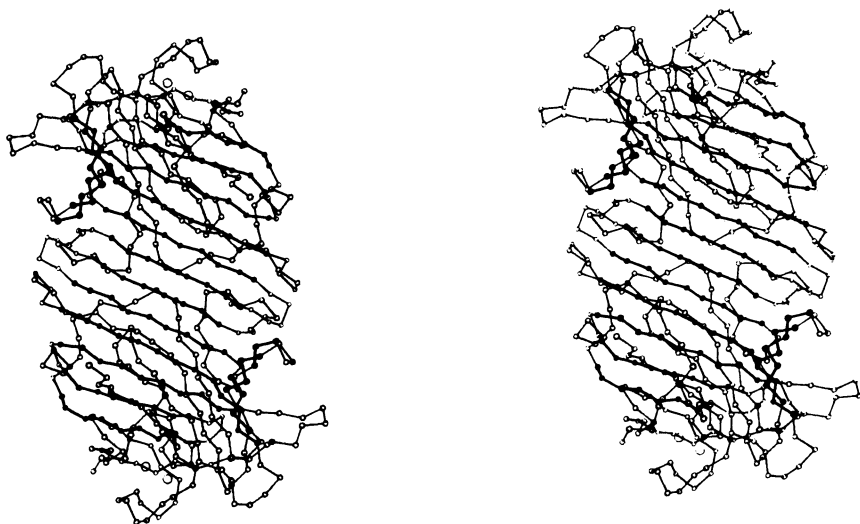


Figure 7. Stereo view of the polypeptide backbone of the Con A dimer with the back β -structure highlighted

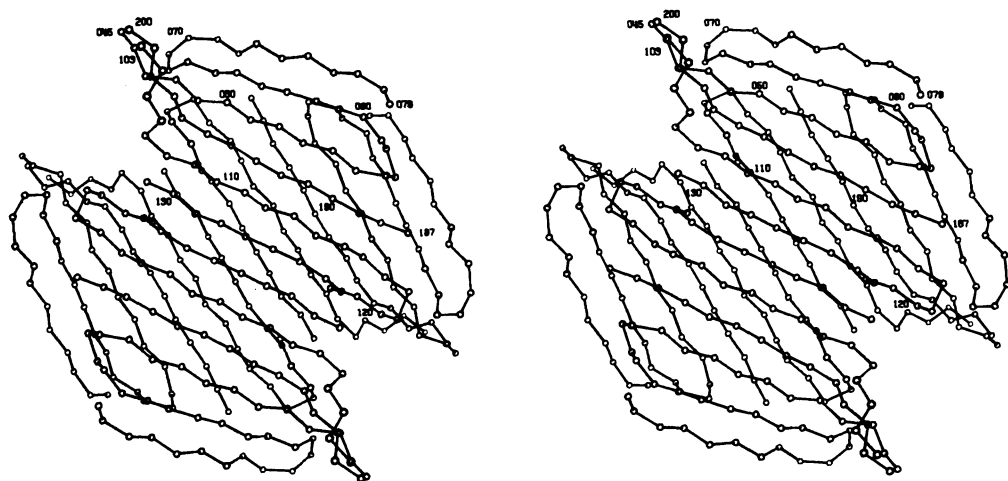


Figure 8. Stereo view of the two back β -structures in the dimers making up a Con A tetramer

114 and Lys 116 on one dimer and Glu 192 on the other dimer. It has been suggested²⁸ that chemical derivatization with compounds such as succinic anhydride may lead to chemical modification of these lysyl residues to prevent tetramer formation and yield a dimeric form of Con A. The interactions between the monomers of the same dimer involve no lysine side chains and would presumably not be affected by these modification procedures.

The data also have suggested a mechanism²⁸ to account for dimer formation at low pH. In the area of dimer-dimer contact His 51 and His 121 are

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sufficiently close to Ser 117 and Ser 108, respectively, to be linked by hydrogen-bonded solvent bridges. The dissociation of the Con A tetramer in acidic solution may result from protonation of the imidazole groups of these histidyl residues, which would in turn alter the hydrogen-bonding patterns.

METAL-BINDING SITES

Con A contains two metal ions, Mn^{2+} and Ca^{2+} . Co^{2+} , Ni^{2+} , Zn^{2+} and Cd^{2+} can replace Mn^{2+} at the transition metal site, but the alkaline-earth site appears to be specific for Ca^{2+} ³⁶. Both metals are lost at low pH²³, and it is possible to crystallize the demetallized protein³⁷. Upon addition of metals to demetallized Con A, it has been shown that the Mn^{2+} ion must be bound before the Ca^{2+} ion, and both metals are required for saccharide binding³⁸. Although transition metals can be bound in the absence of Ca^{2+} , the Ca^{2+} ion has a strong influence on the rate of Mn^{2+} binding³⁹.

X-Ray crystallographic studies^{27, 29, 30} of Con A have provided a detailed description of the ligands and the metal-binding sites. The geometry of the complex is shown in *Figure 9*. The metals are bound near the top of the

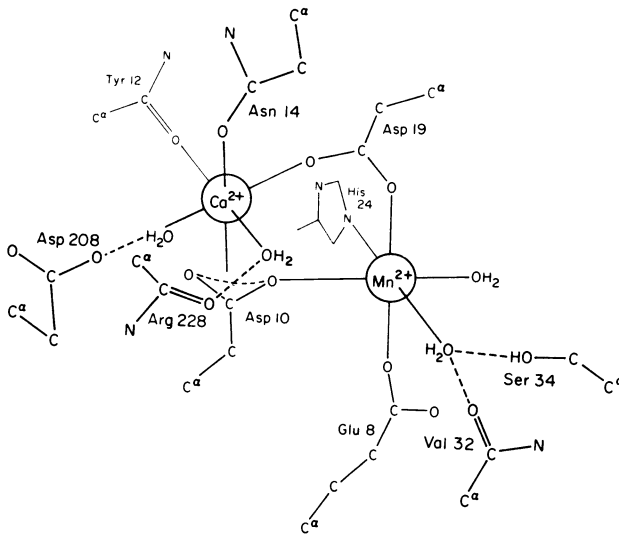


Figure 9. Schematic drawing of the metal-binding sites in Con A

protomer, as shown in *Figure 5*. The two metals are 4.6 Å apart, and each is surrounded by six ligands to form an octahedral coordination shell. For each metal ion, four of the ligands are from the protein and two are water molecules. All of the protein ligands are located in a short segment near the NH_2 terminus of the polypeptide chain. Four of the six ligands for the Mn^{2+} ion are the side chains of Glu 8, Asp 10, Asp 19 and His 24. Of the two ligands that are water molecules, one is hydrogen-bonded to the carbonyl oxygen

of Val 32 and the other is in a solvent-filled channel. The direct ligands for the Ca^{2+} ion are the side chains of Asp 10, Asn 14 and Asp 19 and the carbonyl oxygen of Tyr 12. The two water molecules that serve as ligands for the Ca^{2+} ion are hydrogen-bonded to Asp 208 and Arg 228 near the COOH terminus of the polypeptide chain. Two of the protein ligands, Asp 10 and Asp 19, are common to both metals. The whole arrangement thus forms a binuclear complex of two octahedra sharing a common edge.

The finding that most of the ligands for binding both Mn^{2+} and Ca^{2+} ions are acidic residues accounts for the dependence of metal binding on pH. The fact that Asp 10 and Asp 19 are ligands for both metal ions has suggested²⁹ one explanation for the observation that the transition ion must be bound before the calcium ion. Binding of the Mn^{2+} ion may bring the NH_2 terminal portion of the peptide chain containing the shared ligands into appropriate conformation to create part of the Ca^{2+} binding site. Because the Ca^{2+} ion is bound via two water molecules to the COOH terminal part of the chain, calcium binding would in turn stabilize the conformation of this portion of the molecule. Circular dichroism studies^{32, 40} have indicated that sequential binding of metal ions and saccharide involves conformational changes affecting aromatic residues, although the gross secondary structure of the protein is apparently not affected. Similarly, crystallographic studies³⁷ of demetallized Con A have indicated that it is similar in structure to the native protein, but the geometrical relationships among the subunits are different.

SACCHARIDE BINDING

Most of the biological activities of Con A require binding of the lectin to the cell surface. This binding and, hence, these activities, are inhibited⁹ by simple sugars such as mannose and glucose, but not by closely related stereoisomers such as galactose (*Figure 10*). The specificity requirements for the interaction of Con A with saccharides have been investigated in detail⁴¹ by examining the extent to which monosaccharides, oligosaccharides and modified sugars inhibit precipitation reactions with polysaccharides. The Con A binding site appears to require unmodified hydroxyl groups which can form hydrogen bonds at positions C-3, C-4 and C-6 in the six-membered rings of α -D-glucopyranoside or α -D-mannopyranoside (*Figure 10*). It has been suggested that D-mannose and D-glucose bind to Con A in the C-1 chair conformation and that the protein has a specific site capable of interacting with the anomeric oxygen atom of the α -linked glycosides of these sugars. A β -anomeric oxygen atom interferes with binding in that β -D-mannopyranosides and β -D-glucopyranosides are poor inhibitors. The binding site of Con A can also accommodate certain five-membered rings⁴², such as β -D-fructofuranosides and D-arabinofuranosides, although the binding is considerably weaker than with sugars having six-membered rings.

Two other features of the Con A binding site have come from the inhibition studies: (1) a hydrophobic region is probably close to the saccharide-binding site because phenyl substituents on sugars enhance their inhibitory capacity⁴³; and (2) although the binding probably takes place primarily at the non-reducing termini of polysaccharides, Con A may possess an extended

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binding site capable of interacting with certain non-terminal mannose residues⁴¹.

The specificity of the interactions between Con A and saccharides has been studied in detail, but the amino acid residues participating in the binding of saccharides have not been identified. Direct crystallographic observation of Con A-saccharide interactions has not proved possible as yet. Treatment of Con A crystals with high concentrations of inhibitory

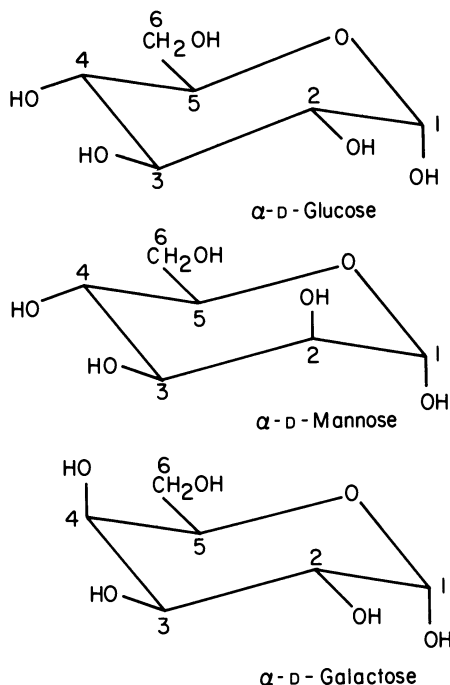


Figure 10. Structures of saccharides that are bound (glucose and mannose) by Con A and a saccharide (galactose) that is not bound by Con A

sugars results in either dissolving of the crystals or loss of the diffraction pattern²⁷. These effects are probably due to conformational changes induced on binding of the saccharides³² and to an increase in the stability of Con A in solution²⁵.

One inhibitory sugar, β -(*o*-iodophenyl)-D-glucopyranoside (β -IPG), does leave a measurable diffraction pattern³³ and shows a peak that can be identified as a bound molecule. The opening that leads to the β -IPG binding site²⁹ (Figure 5) is about 3.5-6 Å wide, 7.5 Å high at the narrowest point and 18 Å deep, widening somewhat at the back. The iodine atom binds at the back of the cavity, about 15 Å from the surface of the protein and 20 Å from the Mn^{2+} ion. The position where the iodine-containing portion of β -IPG is bound is a highly hydrophobic region^{27,44} which in the crystal can also

bind compounds such as iodophenol²⁷. The saccharide portion of β -IPG is not visible but is probably bound closer to the opening of the cavity. Such an orientation would place the saccharide portion near side chains of the protein which are more hydrophilic. This view of the site is consistent with model-building studies and with binding studies, which show that Con A in solution has one binding site per monomer for β -IPG²⁷.

The β -IPG binding site seen in the crystal, however, may not be the same as the inhibitory site in solution. ¹³C nuclear magnetic resonance data⁴⁵ place the saccharide ring carbons of bound α -methylglucoside and β -IPG in nearly identical positions and 10–12 Å from the Mn²⁺ ion, rather than 20 Å from the transition metal as seen in the crystal. In contrast, proton magnetic resonance studies⁴⁶ indicate that the saccharide-binding site is in the position suggested by the x-ray data. The observation that *o*-iodophenol³¹ and β -IPGalactose⁴⁴ bind in the β -IPG binding cavity suggests that the binding specificity is at least less selective in the crystalline state. *o*-Iodophenol and β -IPGalactose do not appear to be bound by Con A in solution³¹, which indicates that there are differences in the binding specificity of the lectin in solution and in the crystal. The carbohydrate-binding site may indeed be in the cavity but its specificity is altered on crystallization. Alternatively, the binding sites present in solution may be lost on crystallization. No conclusions about the actual location of the site can be made, however, until the atomic positions of a bound saccharide are located.

INTERACTION OF CON A WITH LYMPHOCYTES

The accumulated information on the structure and properties of Con A has made it a valuable reagent for analysing interactions at the cell surface. A clear-cut example is the use of Con A to study the mitogenic stimulation of lymphocytes to blast transformation and mitosis^{13,47}. There is evidence⁴⁸ to suggest that the lectin acts directly at the cell surface, inasmuch as covalent coupling of Con A at solid surfaces does not abolish the ability to stimulate cells. Moreover, this protein exhibits a striking cellular specificity in the stimulation of lymphocytes⁴⁸: Con A in solution stimulates thymus-derived (T) lymphocytes, while Con A fixed to solid surfaces stimulates bone marrow-derived (B) cells.

The interaction of lymphocytes with Con A results in a number of immediate structural and biochemical alterations. For example, cell-surface receptors of various types are immobilized^{49,50}. In addition, binding of Con A induces early changes in lipid turnover, in levels of cyclic GMP and in the uptake of small metabolites⁴⁷. Within 24–30 h, synthesis of protein RNA and DNA increases significantly and a large proportion of the cells are transformed into blast cells, with dimensions 2–3 times those of the original small lymphocytes.

Analysis⁵¹ of the kinetics of cellular commitment in the stimulation of mouse lymphocytes by Con A showed that the cells do not all respond in a burst. Rather, with increasing periods of contact with Con A, more and more cells are recruited to lectin stimulation. Once such cells become committed, they synthesize DNA at a rate independent of the length of exposure to the lectin. These results suggest that different cells require different induction periods to be stimulated.

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We have considered two different hypotheses to account for the variability in the induction times required for lymphocyte stimulation. In the first hypothesis we suppose that lymphocytes are heterogeneous and, therefore, different cells differ intrinsically in the length of time that they must be continuously exposed to the mitogen before they are irreversibly stimulated. Alternatively, lymphocytes may be identical cells distributed among several temporal states (perhaps via a cyclic process). In this second model, cells may pass from one state to another but they can only become committed to stimulation when in a particular state in the presence of Con A. A similar hypothesis has been proposed⁵² to explain the stimulatory effects of serum on stationary cultures of chicken fibroblasts. The combined results suggest the possibility that the dispersion of states in G_1 may be a general phenomenon.

In the course of investigating how signals resulting from binding of Con A to the cell surface might be transmitted to the interior of the cell for metabolic stimulation, the distribution and mobility of the cell-surface receptors were analysed in detail. As presented schematically in *Figure 11*, interaction of

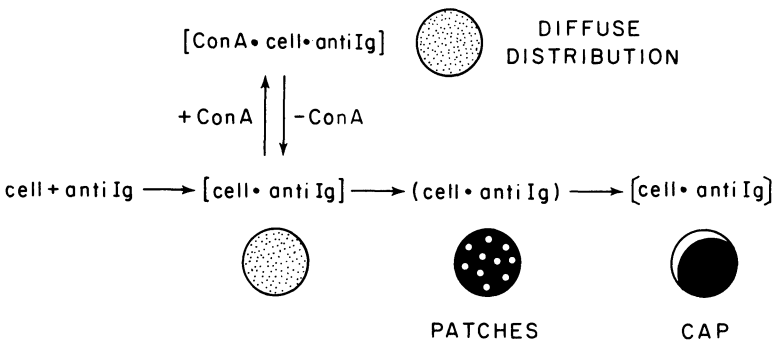


Figure 11. Schematic diagram showing the effects of anti-immunoglobulin and Con A on the distribution of lymphocyte surface receptors. Under certain conditions, Con A can induce cap formation by its own receptors

lymphocytes with fluorescent antibodies against the surface immunoglobulins results first in a diffuse labelling pattern. On further incubation, the immunoglobulin receptors aggregate into 'patches' which in turn coalesce at one pole of the cells to form 'caps'⁵³. Under certain conditions, Con A inhibits both patch formation and cap formation induced with anti-Ig and with antibodies to other receptors^{49, 50}. In contrast to this inhibitory action, Con A itself can induce cap formation by its own receptors on lymphocytes⁴⁹. Con A can thus exhibit two antagonistic properties: (1) the ability to induce cap formation with its own receptors and (2) the ability to restrict the mobility of ligand-receptor complexes such as anti-Ig plus cell surface Ig molecules or Con A plus its glycoprotein receptors.

Although Con A can both induce and inhibit cap formation in lymphocytes, dimeric derivatives of the lectin, such as succinyl-Con A, possess

neither of these activities³⁴. More striking is the observation that both of these activities can be restored by successive treatment of the cells with succinyl-Con A and anti-Con A. Moreover, the monovalent Fab' fragments of anti-Con A do not restore these activities, which suggests that the primary basis for this effect is probably the difference in the valence of the two lectins and their ability to cross-link surface receptors. An alternative explanation, that the differences in the two proteins result from the increased net negative charge of the succinyl-Con A molecule, seems unlikely in light of these experiments but cannot be rigorously ruled out.

The differences in the ability of Con A and succinyl-Con A to alter receptor mobility have permitted some tests of the possible relationship between receptor redistribution and the stimulation of mitosis. For example, Con A can induce cap formation of its receptors, while succinyl-Con A cannot. The two lectins, however, are equally effective mitogens³⁴, which suggests that the process of capping is not required for mitogenic stimulation. In addition, inasmuch as succinyl-Con A also does not inhibit receptor mobility, it appears likely that immobilization of receptors is also not necessary for mitogenesis.

Quantitative analysis of the inhibition by Con A of patch and cap formation on lymphocytes showed striking temperature effects and drug sensitivities. Inhibition of cap formation by Con A occurs when cells are preincubated with Con A at 37° C but not at 4° C. In addition, incubation of cells with colchicine, colcemid or vinca alkaloids suppresses the inhibitory effect of Con A on cap formation. Colchicine and related drugs, as well as low temperatures, are all known to disrupt cellular microtubules, which suggests that inhibition of receptor mobility by Con A might be mediated by microtubules or related colchicine-binding proteins in the cell.

On the basis of these observations, Edelman, Yahara and Wang⁵⁴ have suggested a model for interactions between cell-surface receptors and cytoplasmic structures. This intriguing model postulates that certain surface receptors may be linked to a system of colchicine-binding proteins, and that the mobility of the receptors may be modulated through this anchorage. The hypothesis provides a framework for further investigations into the nature of signalling at the cell surface, and for intercellular communications.

CONCLUSION

Con A is the first lectin for which either the amino acid sequence or the three-dimensional structure has been determined. Structural studies have described the interactions between the subunits and have revealed detailed features of the binding sites of the protein. The primary and three-dimensional structures of Con A provide references for comparison with the structures of other lectins, when these become available. Of particular interest are lectins such as wheat germ agglutinin, which, unlike Con A, has a large number of disulphide bonds, and phytohaemagglutinin (PHA), which shares many of the biological activities of Con A but has a different carbohydrate-binding specificity^{2,7}. The lectin Favin from *Vicia faba* has the same carbohydrate specificity as Con A and has recently been crystallized⁵⁵. Detailed analysis of this protein has been initiated for comparison with Con A. Such

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comparisons may reveal features, such as secondary binding sites, that are not apparent from examination of the structure of a single lectin.

The approaches outlined here for Con A can be extended by the use of other lectins, chemically modified lectins and antibody reagents. The availability of such cell-surface probes promises to expand at the molecular level our understanding of a variety of cellular phenomena, including lymphoid cell stimulation, specific cell-cell interactions, cellular motility and the control of cell growth.

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