# CIRCULAR DICHROISM STUDIES ON CONCANAVALIN A

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Received March 29, 1971

SUMMARY - Optical rotatory dispersion and circular dichroism studies on native and demetallized concanavalin A indicate that this protein consists essentially of a mixture of β-form and random coil. The induction of α-helix in the native protein is accomplished through the use of 2-chloroethanol. The sequential addition of transition metal, $Ca^{2+}$ and α-methyl-D-glucopyranoside to solutions of demetallized protein results in small but reproducible alterations in the aromatic CD spectrum, the largest changes being noted with calcium addition. Aromatic CD difference spectral studies suggest that a single transition may be involved in the binding of metal and α-MG to the protein; the wavelength position of this transition implicates tyrosine residues. At the same time, no significant variations occur in the secondary structure of the protein as reflected by the constancy of the far ultraviolet CD spectrum with ligand complexation. Exploration of the CD aromatic fine structure as a function of pH indicates that both tyrosine and tryptophan chromophores are involved.

Concanavalin A (Con A), a crystalline protein from jack bean, possesses hemagglutining activity (1) as well as the ability to precipitate various polysaccharides such as glycogen, yeast mannan, amylopectin, dextrans (2) and blood serum glycoproteins (3). The native protein contains bivalent metal ions which are essential for its interaction with glycogen (4). Removal of these bivalent metal ions destroys the α-methyl D-glucopyranoside (α-MG) binding sites of this protein (5). The existence of a saccharide binding site in Con A apparently depends on the occupation by $Ca^{2+}$ of a site that itself is formed only when a different metal ion-binding site is occupied by a suitable transition ion.

A preliminary study of the circular dichroism (CD) of Con A has recently appeared (6). The spectrum of the native protein has ellipticity bands which are usually considered to be due to β structure. In addition the pattern between 250 mμ and 300 mμ revealed a complex but distinct fine structure, which was postulated to arise from asymmetric interactions involving tyrosine and tryptophan moieties. Since Con A contains no disulfide bonds there is no complication from the large rotatory strength of this chromophore in the above region.

This paper presents further CD and optical rotatory dispersion (ORD) data on native and demetallized Con A. In particular, CD changes accompanying the sequential addition of transition metal, $Ca^{2+}$ and α-MG are explored in both the aromatic and far UV regions. The aromatic fine structure in the native protein is investigated as a function of pH in order to delineate the chromaphores involved in these transitions. The induction of α-helix in the native protein is accomplished through the use of 2-chloroethanol.

## EXPERIMENTAL

Con A was obtained from Miles-Yeda Ltd. in 30% saturated ammonium sulphate suspension. This protein was 3X crystallized and showed a single band on polyacrylamide gel electrophoresis at pH 4.5. Solutions for experiments were prepared by dialyzing small aliquots of the above suspension against the preferred buffer system, usually 0.2M NaCl, 0.02M sodium acetate, pH 5.2, where the protein is considered to be monomeric (7). Solutions of demetallized Con A ($P_D$) were prepared by the acidification procedure of Kalb and Levitski (8). Representative samples were analysed in a Unicam atomic absorption spectrophotometer and the results were in accord with successful removal of bivalent ions.

The CD and ORD measurements were carried out on a Cary 60 recording spectropolarimeter with attached 6001 CD accessory in accordance with methodology described by Kay (6). The difference spectra were calculated by subtraction of the ellipticities for each of the modified Con A samples from that of the native protein at pH 5.2.

## RESULTS AND DISCUSSION

CD and ORD of Native and Demetallized Con A. The ORD and CD of Con A and $P_D$ were measured at pH 5.2, both in the visible and UV regions. Fig. 1 illustrates a UV ORD spectrum which is typical of either Con A or $P_D$. This pattern is characterised by a trough with a minimum at 230.5 mμ $[m^1]_{230.5} = -2700°$, a cross-over point at 224 mμ and a peak at 209.5 mμ

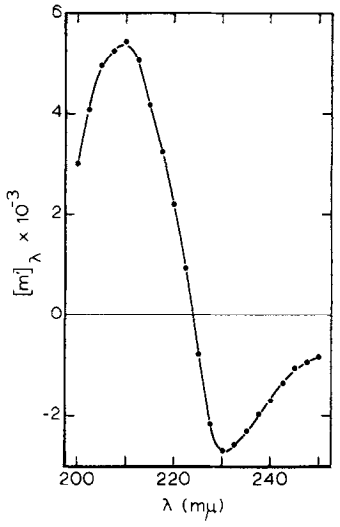


Fig. 1 - The far ultraviolet ORD spectrum of native or demetallized concanavalin A in 0.2M NaCl, 0.02M sodium acetate, pH 5.2.

with $[m^1]_{209.5} = 5400°$. These parameters would suggest that the native polypeptide chain consists of a mixture of β structure and random coil (9). The visible ORD parameters were $a_o = -220°$ (±15°), $b_o = 0$ (±10°) and $\lambda_c = 217$ (±1) mμ, indicating essentially the absence of α-helix. Application of the empirical relationship of Timasheff (10) to the visible ORD data results in the calculation of some 30% β structure in the native molecule.

The aromatic CD characteristics for the native protein at pH 5.2 and 7.5 and for $P_D$ at pH 5.2 are demonstrated in Fig. 2. In agreement with the work of Kay (6), these spectra consist essentially of three positive bands at 291, 283, and 265 mμ with ellipticities of the order of 30-100 deg. $cm^2$ $decimole^{-1}$. Two points are worthy of note here. Firstly, the spectrum of $P_D$ at pH 5.2 is more positive throughout the region compared with native Con A, and secondly, the pattern for Con A at pH 7.5 is more negative than the control at pH 5.2. It should be mentioned that the far UV spectra for these three samples showed only very minor variations. The differences between samples in the aromatic region must reflect local environmental differences in the vicinity of tyrosine and tryptophan residues rather than gross alterations in protein secondary structure.

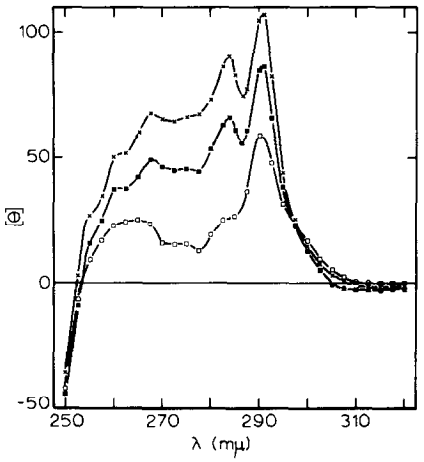


Fig. 2 - Molar ellipticity values in the aromatic region for native Con A at pH 5.2 (■-■), native Con A at pH 7.5 (□-□) and demetallized protein at pH 5.2 (x-x).

CD Changes with the Sequential Addition of Transition Metal, $Ca^{2+}$ and α-MG to $P_D$. The changes noted in the CD pattern in the aromatic absorption region by sequential addition of $Ni^{2+}$, $Co^{2+}$, or $Mn^{2+}$, then $Ca^{2+}$ and finally α-MG to $P_D$ solutions are presented in Fig. 3. Upon the addition of any of the above three transition metal ions, the spectrum below 285 mμ becomes more positive, although the band positions are unchanged. There is essentially no alteration in the magnitude of the 291 mμ band. If $Ca^{2+}$ is now added to these solutions there is approximately a 50% decrease in the complex band area below 285 mμ, and the 291 mμ band diminishes by about 20%. Addition of carbohydrate produces a spectrum very similar to that of $P_D$. It should also be noted that these changes in the aromatic absorption region are accompanied by essentially no alteration in the far UV CD spectrum, suggesting again that the processes involve side chain interactions without affecting the overall protein secondary structure.

Con A contains two different metal binding sites, the transition metal binding site ($S_1$) and the $Ca^{2+}$-binding one ($S_2$) (5). $P_D$ contains the transition metal site only. The $Ca^{2+}$-binding site is formed when a transition metal occupies $S_1$ whereas the saccharide binding site is formed when $S_2$ is

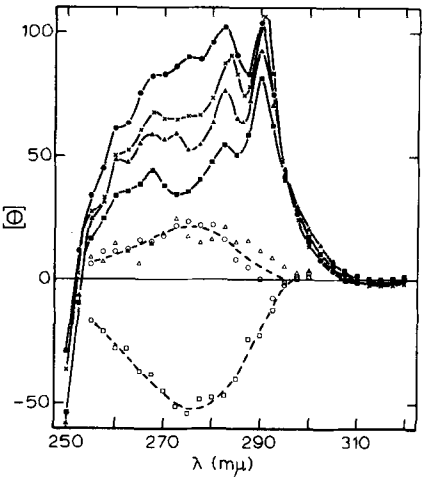


Fig. 3 - Molar ellipticity values in the aromatic region for demetallized protein ($P_D$) at pH 5.2 (x-x), $P_D$ + $10^{-3}$ M transition metal ion (●-●), $P_D$ + $10^{-3}$ M transition metal + $10^{-3}$ M $Ca^{2+}$ (■-■), $P_D$ + $10^{-3}$ M transition metal + $10^{-3}$ M $Ca^{2+}$ + $10^{-3}$ M α-Methyl-D-glucopyranoside (▲-▲). Also included are difference spectra: $P_D$ + transition metal - $P_D$ (o-o), $P_D$ + transition metal + $Ca^{2+}$ - [$P_D$ + transition] (□-□), $P_D$ + transition + $Ca^{2+}$ + MG - [$P_D$ + transition + $Ca^{2+}$] (Δ-Δ). Transition metal in this case was $Ni^{2+}$.

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occupied by $Ca^{2+}$. Electron spin resonance (ESR) studies (11), suggest that $Mn^{2+}$ is covalently bound, and when $Ca^{2+}$ is bound at the $S_2$ site, spectral changes indicate an alteration in the environment of the transition metal site. Binding of α-MG to the saccharide binding site causes no change in the ESR spectrum.

The changes induced in the aromatic CD spectra are in good agreement with the above postulated mechanism and experimental observations. For example, the largest alterations occur with addition of $Ca^{2+}$ and further, when the sugar is bound, the spectrum is very similar to $P_D$. It is noteworthy that the difference CD curves (inset, Fig. 3), generated as indicated in the figure legend, are identical and positive for both $Ni^{2+}$ and α-MG addition with a $\lambda_{max}$ for the peak at 275 mμ, while the difference curve generated for $Ca^{2+}$ is negative with a $\lambda_{min}$ for the trough also at 275 mμ. The virtual identity of the wavelength of the transition in the three cases is indicative of a similar

chromophoric group being involved, and the wavelength position of the transition, 275 mμ, is suggestive of local tyrosine residues (12) being implicated in the binding of metal and α-MG to the protein, without modification of the protein secondary structure. A similar involvement of tyrosine residues in the binding of metals such as $Fe^{2+}$, $Mg^{2+}$ and $Cu^{2+}$ to conalbumin has been reported (13).

The Effect of pH. In order to delineate the various chromophores contributing to the aromatic spectrum, this region was explored as a function of pH (Fig. 4). It is to be noted that Con A is very pH sensitive undergoing aggregation when the pH is raised above 5.8 (7). The protein possesses its maximum binding affinity at pH 7, presumably when the molecule is in an aggregated form. The changes shown in Fig. 4 are time dependent and intermediate states can be observed from zero time up to two or three hours at the chosen pH value. Most of the data presented, with the two exceptions noted, were measured after protein exposure for some twenty-four hours at the indicated pH.

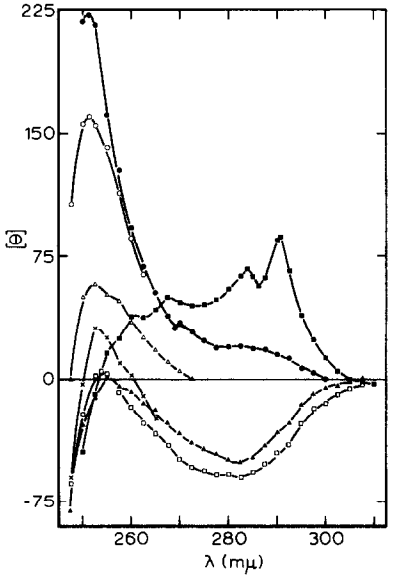


Fig. 4 - Molar ellipticity values in the aromatic region for native Con A as a function of pH: pH 5.2 (■-■), pH 9.32 (▲-▲), pH 10.06 (□-□), pH 10.6 (x-x), pH 10.85 for 2 hours (Δ-Δ), pH 11.4 for 2 hours (o-o), and pH 13.0 (●-●).

As the pH increases, the complex band structure (265 mμ - 291 mμ) decreases, with generation of a positive peak at approximately 249 mμ, the magnitude of which increases with pH. Since there are no disulfide bridges in the molecule, this band must be due to ionized tyrosines, a conclusion well substantiated from comparable studies with model compounds (14), bromelein (15) and lysozyme (16).

The decrease in the magnitude of the 291 mμ band is more difficult to rationalise in terms of groups involved. The charge alteration and unfolding produced by elevating the pH may be changing the environment of both tyrosine and tryptophan residues giving rise to the observed new negative ellipticity band at 282 mμ.

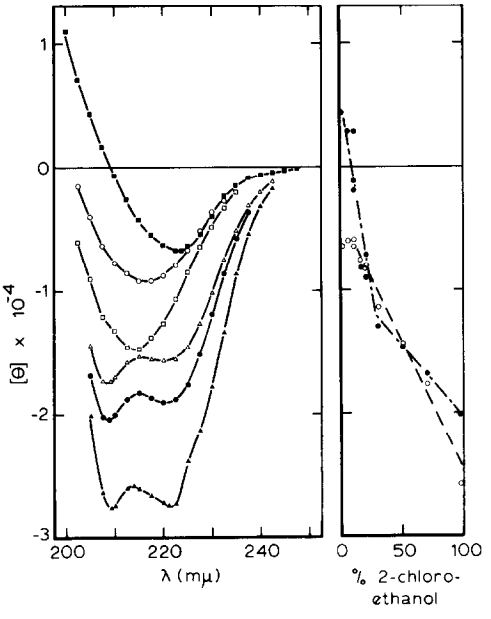


Fig. 5 - Molar ellipticity values in the far UV region for native Con A in increasing concentrations of 2-chloroethanol: 0% (■-■), 10% (o-o), 20% (□-□), 50% (Δ-Δ), 70% (●-●), 97.5% (▲-▲). The inset shows change in $[\theta]_{205}$ (●-●) and $[\theta]_{225}$ (o-o) with 2-chloroethanol concentration.

The Effect of 2-Chloroethanol. The effect of the helix promoting solvent 2-chloroethanol on the far UV circular dichroism spectrum of Con A is shown in Fig. 5. The inset in this figure documents the change in the magnitude of $[\theta]_{205}$ and $[\theta]_{225}$ values as a function of perturbant concentration.

As the concentration of 2-chloroethanol is raised from 0 - 30%, the 222.5 mμ trough undergoes a blue shift to about 215 mμ. As the concentration of organic solvent is further increased, the doublet structure characteristic of α-helical proteins becomes evident. It is also noted that $[\theta]_{205}$ decreases rapidly up to ∿30% 2-chloroethanol and then undergoes a small linear decrease up to 97.5% perturbant. $[\theta]_{225}$ on the other hand displays a slight increase from 0 - 10%, and then shows a gradual linear decrease up to 97.5% organic solvent.

The hydrodynamic and spectral measurements of Weber and Tanford (18) suggest that the path of a protein from the native state in water to that in 2-chloroethanol may involve a structurally disorganized intermediate(s). Additionally, 2-chloroethanol has the ability to promote exposure of the tyrosine and tryptophan residues of proteins to an extent approximately equivalent to 8M urea (18).

By analogy with the above studies, the Con A data presented indicate that at 2-chloroethanol concentrations up to about 30% the principle effect is the destruction of the β form resulting in a more disorganized structure, with the formation of α-helix proceeding at a much slower rate. As the concentration of organic solvent increases further, the α-helix becomes the preferred

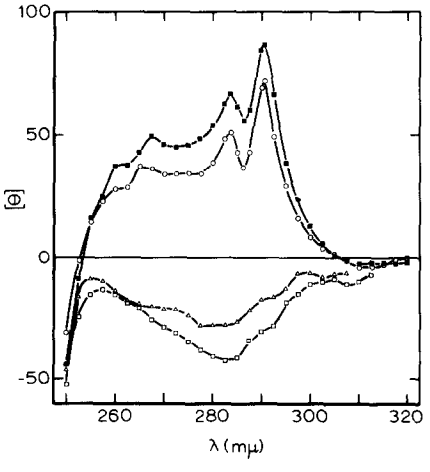


Fig. 6 - Molar ellipticity values in the aromatic region for native Con A in 0% (■-■), 10% (o-o), 15% (Δ-Δ) and 20% (□-□) 2-chloroethanol.

conformation and consequently its CD characteristics become more pronounced.

Turning to the 250 - 300 mμ region it is noted that the effects of 2-chloroethanol are perhaps more dramatic (Fig. 6). Between 10 - 15% concentration of perturbant there is complete elimination of the 291 and 283 mμ bands and generation of a new negative band at 283 mμ. No change in the magnitude of this band occurs above 20% 2-chloroethanol. These alterations presumably arise from the "exposing" effect of the organic solvent on tyrosine and tryptophan residues. The local environment in the neighbourhood of these groups is subtly changed resulting in the generation of the new negative ellipticity band at 283 mμ.

ACKNOWLEDGEMENT

This investigation was generously supported by the Medical Research Council of Canada (MRC-MT-1223), the Life Insurance Medical Research Fund and the Muscular Dystrophy Association of Canada.

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