

THE EFFECT OF BINDING OF METAL IONS ON THE THREE-DIMENSIONAL STRUCTURE OF DEMETALLIZED CONCANAVALIN A

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1. Introduction

Concanavalin A (con A) is a saccharide-binding protein of the Jack bean [1]. The protein contains two different metal-binding sites, which must be occupied in order for saccharide binding to be possible [2,3]. When the metal ions are removed by acid treatment, the demetallized protein can bind only divalent transition metal ions (Mn^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , or Cd^{2+}) at a site designated S1, but it cannot bind Ca^{2+} or saccharide. When S1 is occupied by one of the metal ions listed above, the protein can bind Ca^{2+} at a second metal-binding site designated S2 [4].

We have made an X-ray crystallographic determination of the three-dimensional structure of demetallized concanavalin A and we report here the important structural differences in the metal-binding region between the demetallized protein and the native metalloprotein [5–7] (fig.1). We postulate a detailed mechanism whereby binding of the transition metal ion at S1 engenders the Ca^{2+} -binding site (S2). The experimental details, refinement procedure and electron density maps upon which our structure is based will be reported fully [8].

2. Experimental

Con A was prepared from Jack-bean meal (Sigma

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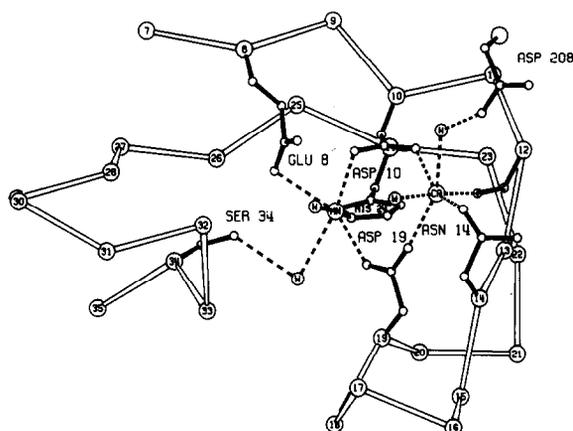


Fig.1. Schematic view of the Mn^{2+} - and Ca^{2+} -binding region in native con A (based on coordinates of [6]). The open lines represent virtual bonds between successive alpha-carbon atoms of residues 7–35. The side-chain ligands are shown in solid lines, and the coordination of the metal ions in dashed lines. Water molecules are designated 'W'. Note that all the direct protein ligands are within the loop of residues 8–24.

Chemical Co., St. Louis, MO) by the method in [9] and the metal ions were removed by acid treatment [3]. The metal ion content, as determined by atomic absorption spectroscopy (Perkin Elmer, 303 instrument) was less than 0.02 Zn atoms/subunit and less than 0.02 Mn atoms/subunit. The protein was crystallized as in [10] from 1.65 M $(NH_4)_2SO_4$ in 0.05 M sodium acetate buffer, pH 5.0, containing 0.2 M NaCl. The space group is orthorhombic, $P2_1 2_1 2_1$, with unit-cell dimensions $a = 84.3 \text{ \AA}$, $b = 91.2 \text{ \AA}$, $c = 61.1 \text{ \AA}$,

and there are 2 protomers/asymmetric unit. Some 8000 unique reflections, extending to a resolution of 3.2 Å, were measured on a CAD-3 automatic diffractometer, using Ni-filtered Cu K α radiation. Phases were determined by molecular replacement on the basis of published coordinates for native con A [6]. The resulting electron density map was difficult to interpret in the region of the metal-binding sites which suggested significant differences between the native and demetalized structures, particularly in that region. Systematic adjustment of the structure by means of the CORELS reciprocal space least-squares procedure [11] led to a clean and generally interpretable map and reduced the *R*-factor from 47–26%.

3. Results

The structure of demetalized con A is generally very similar to that of native, metal-containing con A. However, there are several conformational differences, more clearly seen in protomer I, in the region of the metal ligands (see fig.2):

1. In the backbone loop of residues 7–25 there is a slight, yet consistent, closing up around the positions occupied by metal ions in the native form.
2. The carboxyl group of Asp 19 is ~ 2 Å further away from the calcium site, but ~ 1.5 Å closer to a nitrogen atom of the imidazole ring of His 24.
3. The side chain of Glu 8, beyond the γ -carbon atom, is invisible in the electron density map, probably because of dynamic disorder.

These changes are also observed for protomer II except that Asp 19 is not shifted further away from the calcium position, but only gets closer to His 24. This difference between protomers I and II appears to be caused by packing constraints (see [8]).

4. Discussion

The positions of Asp 10, Asp 19 and His 24 suggest an explanation for the inability of the demetalized protein to bind Ca $^{2+}$. In the native protein, the transition metal ion in S1 is complexed to carboxyl oxygens from Glu 8, Asp 10 and Asp 19 and to a nitrogen from the imidazole ring of His 24 (fig.1). The carboxyl residues of Asp 10 and Asp 19 constitute bridges between the transition-metal ion of S1 and Ca $^{2+}$ of S2. The large separation between the carboxyl of Asp 19 and that of Asp 10 in the demetalized protein, as seen in fig.2, renders this pair of potential Ca $^{2+}$ ligands ineffective. Evidently, a single carboxyl ligand is insufficient to create a specific Ca $^{2+}$ -binding site, as there are indeed many isolated carboxyl groups on the surface of concanavalin A and in other proteins which do not bind Ca $^{2+}$ ions appreciably or specifically. Asp 19 appears to be held in an unfavorable position for ligand pairing with Asp 10 by virtue of a salt-bridge with His 24. Such an interaction would be favored at pH 5 since the imidazole ring would be positively charged and the carboxyl group negatively charged. Binding of a metal ion at S1 deprotonates the histidine [12]. Consequently, the salt-bridge with

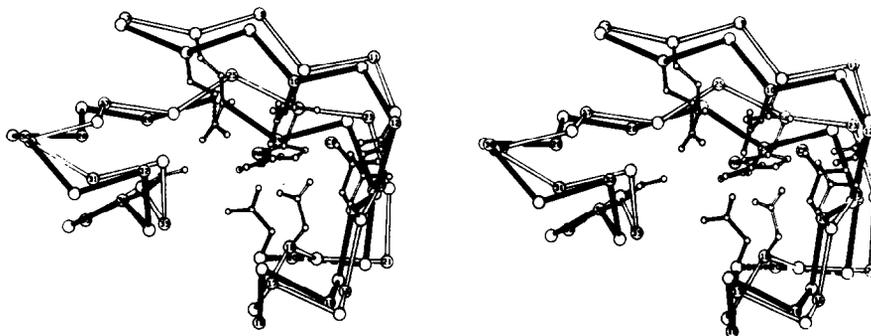


Fig.2. Stereo representation of residues 7–35 in demetalized and native con A viewed down the *z*-axis. The backbone is represented by virtual bonds between successive alpha-carbons. Details are shown of ligands which participate in metal binding, including the carbonyl group of Tyr 12 and side chains of Glu 8, Asp 10, Asn 14, Asp 19, His 24 and Ser 34. Flexible parts are shown in dashed lines. Protomer I (solid lines) and native (open lines).

Asp 19 would be broken and the carboxyl group would be free to assume a position where it could form a favorable binding site for Ca^{2+} with the carboxyl of Asp 10. In the absence of a transition metal ion, Glu 8 is free to move and is therefore not seen in the electron density map. When the transition metal is bound, however, this carboxyl group should be fixed by coordination with it.

More complicated equilibria between several conformational states of con A in solution have been postulated recently on the basis of magnetic resonance data [13]. The work presented here does not rule out those possibilities. These authors [13] also speculate about a *cis-trans* isomerization of a peptide unit upon demetallization of con A. The peptide linkage between Ala 207 and Asp 208 is in the *cis*-configuration in native con A [6,7], and our results are consistent with such a *cis*-peptide in the demetallized form, too. However, at the resolution obtainable from these crystals it is difficult to rule out a *trans*-configuration. In another recent crystallographic study of the same crystal from of demetallized con A, such a *cis-trans* isomerization has been suggested [14]. These authors reported difficulty in interpreting their maps in the metal-binding region (residues 12–22). This may be due to a higher degree of disorder in their crystals or to the incomplete refinement of their data ($R = 33.49\%$).

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