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High-Resolution Structures of Single-Metal-Substituted Concanavalin A: the Co,Ca-Protein at 1.6 Å and the Ni,Ca-Protein at 2.0 Å

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Abstract

The molecular structures of cobalt- and nickel-substituted concanavalin A have been refined at 1.6 and 2.0 Å resolution, respectively. Both metal derivatives crystallize in space group *I*222 with approximate cell dimensions $a = 89$, $b = 87$ and $c = 63$ Å and one monomer in the asymmetric unit. The final R factor for Co-substituted concanavalin A is 17.8% for 29211 reflections with $F > 1.0\sigma(F)$ between 8.0 and 1.6 Å. For Ni-substituted concanavalin A the final R factor is 15.9% for 16128 reflections with $F > 1.0\sigma(F)$ between 8.0 and 2.0 Å resolution. Both structures contain a transition-metal binding site and a calcium-binding site but, unlike Cd-substituted concanavalin A, do not have a third metal-binding site. The Co-substituted concanavalin A structure diffracts to the highest resolution of any concanavalin A structure reported to date. A comparison of the structures of Ni-, Co-, Cd-substituted and native concanavalin A gives an indication of coordinate errors, which is a useful baseline for comparisons with saccharide complexes of concanavalin A described in other work. We also give a detailed account of multiple conformations which were found for five side-chain residues.

1. Introduction

Concanavalin A is a saccharide-binding protein from the Jack bean (*Canavalia ensiformis*) which belongs to the class of proteins known as lectins. Due to its carbohydrate-binding properties it is widely applied in biological and biochemical research (Bittiger & Schnebli, 1976).

It had been shown as early as 1936 that saccharide binding by concanavalin A depends on the presence of divalent metal ions (Sumner & Howell, 1936). Later it was demonstrated that concanavalin A has two distinct metal-binding sites and that both must be occupied for saccharide binding to occur. The first metal-ion site of concanavalin A (*S*1) specifically binds the transition metals Ni, Co, Zn, Mn and Cd whilst the second site (*S*2) binds only Ca and Cd, which are of similar ionic radius (Kalb & Levitzki, 1968; Yariv, Kalb & Levitzki, 1968; Shoham, Kalb & Pecht, 1973). Comparison with the structure of demetallized concanavalin A suggests that the transition-metal site *S*1, which is close to the Ca-binding site *S*2, directly generates the ligand geometry around the second metal site (Shoham *et al.*, 1979). The metal ions serve to hold the polypeptide chain in place for sugar binding to occur (Derewenda *et al.*, 1989; Harrop *et al.*, 1993). As isolated from the Jack bean, concanavalin A contains a mixture of transition metals at site *S*1. The site *S*2 is occupied by Ca ions. The metals can be removed in acidic media at pH 1.2; the protein can be reconstituted in the presence of calcium and the chosen transition metal at pH 5.2.

The structure of this metalloprotein has been of interest since an *I*222 crystal of the saccharide-free concanavalin A was described (Greer, Kaufman & Kalb, 1970). In the *I*222 crystal form concanavalin A was shown to be a tetramer with one monomer of 237 amino acids in the asymmetric unit (approximate

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cell dimensions: $a = 88$, $b = 86$ and $c = 62$ Å). The first detailed decription of the metal-binding region in native concanavalin A at 1.75 Å resolution was given by Hardman, Agarwal & Freiser (1982).

The quality of the *I*222 crystals improves when the protein is reconstituted from demetallized concanavalin A with a single metal ion in the transition-metal site *S*1 (metal = Co, Ni, Zn, Mn or Cd) and Ca in *S*2 [Kalb (Gilboa), Yariv, Helliwell & Papiz, 1988]. The diffraction limit of these single transition-metal crystals was determined by exposure to monochromatic synchrotron radiation. That work pointed to a correlation between the resolution limit of the crystals and the radius of the transition-metal ion, each of these derivatives diffracting to a higher resolution than native concanavalin A. So far, only the Cd-substituted concanavalin A has been studied at high resolution (Naismith *et al.*, 1993). In that metal derivative a second Cd ion occupies an additional metal site (*S*3), which is located at the dimer interface, so that two Cd ions form a bridge between the two monomers. It has been shown that the site *S*3 also binds Pb^{2+}, Sn^{3+} (Becker, Reeke, Wang, Cunningham & Edelman, 1975) and is apparently less specific in its metal requirement than the sites *S*1 and *S*2.

In this paper we present two high-resolution structures of concanavalin A where the transition-metal site of concanavalin A is occupied by Co^{2+} or by Ni^{2+}. We also compare these two structures with the structures of native and Cd-substituted concanavalin A, these latter being at a resolution of 2 Å. The study of these single transition-metal derivatives allows the effects of metal substitution on the protein to be assessed and contributes to our understanding of the third metal-binding site. The Co-substituted concanavalin A structure is the highest resolution structure (1.6 Å) reported of any crystal form of concanavalin A or of any lectin.

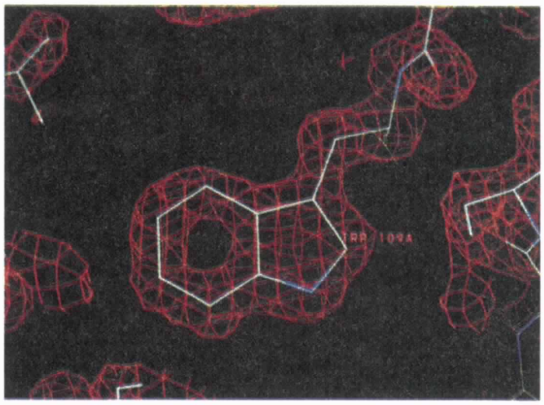
2. Methods

2.1. *Data collection for* Co-*substituted concanavalin A*

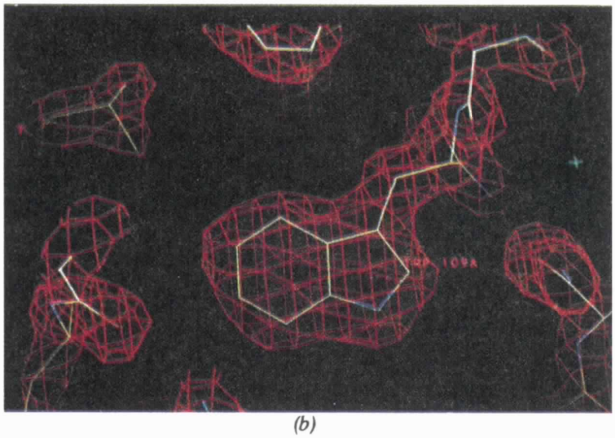
Crystals of Co-substituted concanavalin A were grown as described previously [Kalb (Gilboa), Yariv, Helliwell & Papiz, 1988]. Data to 1.6 Å resolution were collected on beamline X31 at the EMBL outstation at DESY in Hamburg (Wilson, 1989), using synchrotron radiation of wavelength 1.009 Å and a MAR Research image-plate area detector. One crystal of dimensions 1.2 × 0.8 × 0.4 mm was used to collect the X-ray intensity data. A total of 30245 unique reflections were measured from 90 1° oscillation images. The cell dimensions were found to be $a = 88.7$, $b = 86.5$ and $c = 62.5$ Å. The data were processed using an adapted version of the *MOSFLM* suite of programs (Wonacott, Dockerill & Brick, 1980). Missing data, up to 3.0 Å resolution, were then measured from a new crystal on a Rigaku four-circle diffractometer set up on a Cu $K\alpha$ rotating-anode with a graphite monochromator. The two data sets were scaled together using *ANSC* (*CCP*4, SERC Daresbury Laboratory, 1979). The mean fractional isomorphous difference on F was 7.6% for 3826 common reflections up to 3.0 Å. Table 1(*a*) summarizes the final data set used for the refinement. The merging R value for the synchrotron data only was 4.8% (on intensity). The completeness of the merged data [$F > 1\sigma(F)$] was 91.9%.

2.2. *Data collection for* Ni-*substituted concanavalin A*

A single crystal with approximate dimensions 1.5 × 0.7 × 0.3 mm was used to collect data to 2.0 Å resolution on an R-AXIS IIC image-plate system mounted on a Rigaku RU-200 Cu $K\alpha$ rotating-anode generator, operated at 50 kV and 100 mA.



(*a*)



(*b*)

Fig. 1. The electron density at residue Trp109, $2F_o - F_c$ map contoured at two times the r.m.s. of the electron density observed in the unit cell illustrating the benefit of the 1.6 *versus* 2.0 Å data; (*a*) Co-concanavalin A structure, (*b*) Ni-concanavalin A structure.

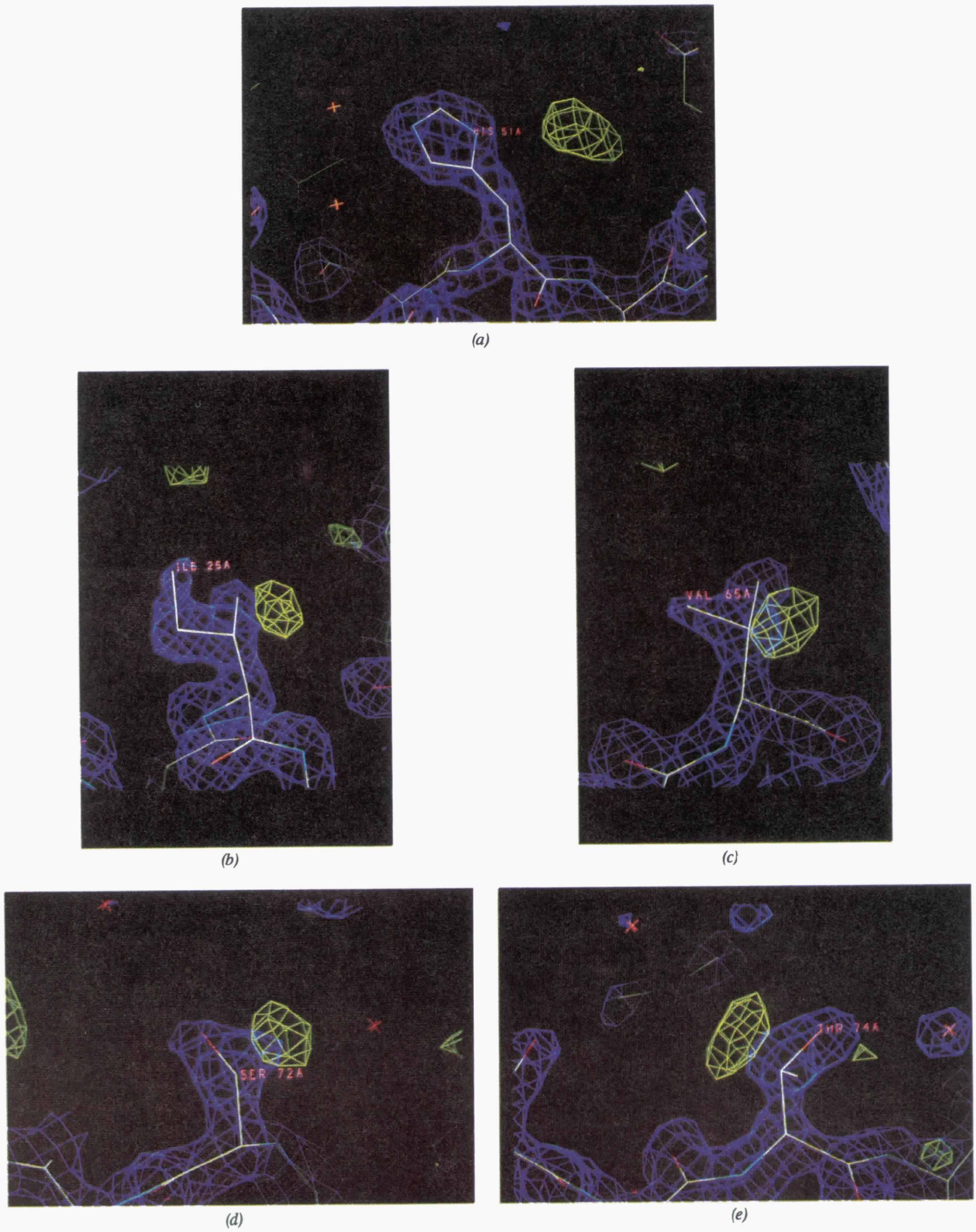


Fig. 2. Multiple conformations at (*a*) His51, (*b*) Ile25, (*c*) Val65, (*d*) Ser72 and (*e*) Thr74 taken from the Co-substituted concanavalin A refined structure ($2F_o - F_c$ map contoured at 2 r.m.s., blue; $F_o - F_c$ map contoured at 3σ, green).

Table 1. *X-ray crystallographic data*

Resolution (Å)	All data No. of reflections	All data Completeness (%)	Data with $F > 1.0\sigma(F)$ No. of reflections	Data with $F > 1.0\sigma(F)$ Completeness (%)
(*a*) Cobalt-substituted concanavalin A				
8.00–4.00	1888	99.7	1850	97.7
4.00–3.02	2751	99.6	2615	94.7
3.20–2.53	2940	86.5	2917	85.9
2.53–2.22	3506	89.9	3506	89.9
2.22–2.00	3984	91.1	3984	91.1
2.00–1.84	4410	92.1	4410	92.1
1.84–1.71	4804	92.8	4804	92.8
1.71–1.60	5125	93.3	5125	93.3
8.00–1.60	29408	92.5	29211	91.9
(*b*) Nickel-substituted concanavalin A				
8.00–4.72	1091	99.8	1087	99.7
4.72–3.67	1483	99.9	1473	99.3
3.67 3.11	1811	99.9	1796	99.1
3.11–2.74	2040	99.8	2013	98.6
2.74–2.48	2288	99.7	2232	97.5
2.48–2.29	2491	99.6	2432	97.6
2.29–2.13	2672	99.4	2557	95.7
2.13–2.00	2838	93.2	2538	89.4
8.00–2.00	16474	98.6	16128	96.4

The image plate was set at a distance of 97 mm from the crystal. The refined cell dimensions were $a = 89.4$, $b = 87.2$ and $c = 63.1$ Å. A total of 60304 observations [$F > 1\sigma(F)$] were measured from 50 oscillation frames at 1.8° intervals and 50 min exposure per frame. The data reduction and scaling were performed with the program package *PROCESS* (Higashi, 1990). The unweighted merging R value was 4.0% with 16435 independent reflections greater than $1.0\sigma(I)$ to a resolution of 2.0 Å, which represents 97.4% of the total possible reflections at that resolution (see Table 1*b*).

2.3. *Refinement of the* Co-*substituted concanavalin A structure*

The refinement was carried out using *X-PLOR* Version 2.1 (Brünger, 1990). The starting model was based on the refined coordinates of the Cd-substituted concanavalin A structure (Naismith *et al.*, 1993; PDB reference file name: 1CON). All solvent molecules and the Cd ion occupying the third metal site were removed prior to the first refinement stage and all temperature factors were reset to 20.0 Å^2. Initial refinement was carried out using reflections in the 8–2 Å resolution shell. The resolution shell was expanded in stages to 8–1.8 Å and then to 8–1.6 Å. Data with $F > 1\sigma(F)$ only were included. Temperature factors were restrained for the first stages of refinement only. No metal–ligand bond-distance restraints were employed and the metal ions were given no charge. Each refinement stage comprised several cycles of positional (*POWELL* minimization option of *X-PLOR*) and temperature-factor refinement. The correctness of the model was checked using *PROCHECK* (Laskowski, MacArthur, Moss & Thornton, 1993), some of the side chains were adjusted and water molecules located using $2F_o - F_c$ and $F_o - F_c$ electron-density maps (SERC Daresbury Laboratory, 1979) calculated at various stages. Rebuilding of the model was carried out on an Evans and Sutherland ESV30 using *FRODO* (Jones, 1978). Water molecules were inserted in the model if they formed at least one stereochemically reasonable hydrogen bond and were above the 3σ level in difference-density maps; they were deleted if they failed to reappear at the 1 r.m.s. level of the $2F_o - F_c$ density in the unit cell. *PEAKSEARCH* and *WATERSORT* were employed to locate the final water molecules (SERC Daresbury Laboratory, 1979). The differences between the initial and the final model are discussed later. The final R factor was 17.8% for 29211 reflections with $F > 1\sigma(F)$ in the 8–1.6 Å resolution shell.

2.4. *Refinement of the* Ni-*substituted concanavalin A structure*

The coordinates of the Co-substituted concanavalin A were used to start the refinement, which was again carried out with *X-PLOR* Version 2.1 (Brünger, 1990). All reflections in the resolution range 8–2 Å with $F > 1\sigma(F)$ were employed. The model was initially subjected to ten cycles of rigid-body refinement which indicated small translational shifts (0.45 Å in a, 0.25 Å in b and 0.1 Å in the cell c direction) and a rotational shift or less than 0.1°. All other details of the refinement are essentially as described in §2.3. The final R factor was 15.9% for reflections with $F > 1\sigma(F)$ in the 8.0–2.0 Å resolution range.

2.5. *Accuracy of the* Co-*substituted concanavalin A structure*

The final model contains 149 water molecules with one of them on a special position; one calcium and

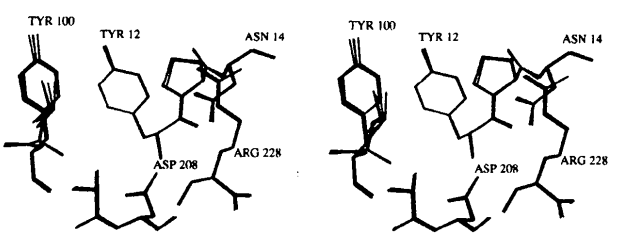


Fig. 3. Superposition of the Co-, Ni-, Cd-substituted and native concanavalin A refined structures in the saccharide-binding site, in stereo, to illustrate the high reliability structural comparisons have in this functionally important region of the molecule (see also Table 4).

one cobalt ion were identified at sites *S*2 and *S*1, respectively. There was no evidence of a cobalt at the metal site *S*3 which was occupied in the cadmium structure (Naismith *et al.*, 1993). Some stereochemical parameters of the final model are given in Table 2.

A Ramachandran plot (Ramakrishnan & Ramachandran, 1965) suggests that all non-glycine residues lie within the allowed regions, 87.5% of these residues are found in the most favourable regions. From a Luzzati plot (Luzzati, 1952) the average positional error lies in the range from 0.15 to 0.20 Å.

The electron-density maps are very clear. The high quality of the electron-density map becomes obvious when looking at aromatic side chains; some of them show characteristic central holes in the six-membered rings at the 2 r.m.s. level of the $2F_o - F_c$ electron-density map in the unit cell (Fig. 1). All atoms were in at least 0.5 r.m.s. $2F_o - F_c$ density. Main-chain breaks at the 1 r.m.s. level of the $2F_o - F_c$ electron-density map occurred at poorly defined loop regions around residues 120 and 160. The highest peak in the $F_o - F_c$ map (6σ) occurred near the side chain of His51 and would appear to be a partially occupied conformation of this side chain (Fig. 2). When modelling both conformations of His51, the occupancy for the major conformation was 0.7 and for the minor conformation the occupancy was 0.3. The corresponding values for the Ni structure are essentially the same. As the minor conformation of His51 did not reappear at the 1 r.m.s. level of the $2F_o - F_c$ map, we have not included it in the coordinate file submitted to the Protein Data Bank. Nevertheless, attention is drawn here to the evidence for these. Likewise multiple side-chain conformations are also observed for Ile25, Val65, Ser72 and Thr74 (Fig. 2).

The *B* values also provide a measure of the quality of the structure. In the present structure, the average *B* values for all protein atoms and all water molecules are 17.8 and 30.9 Å^2, respectively. The corresponding values for the main-chain and side-chain atoms are 13.6 and 20.2 Å^2, respectively (Table 3). These values are slightly lower than those for the native and the Ni- and Cd-substituted concanavalin A structures (Table 3), which are at a resolution of 2.0 Å.

2.6. *Accuracy of the* Ni-*substituted concanavalin A structure*

The final model of the Ni-substituted concanavalin A structure comprises 144 water molecules (one on a special position), one calcium and one nickel ion; no third metal site could be identified.

The stereochemical quality of the model is similar to the Co-substituted concanavalin A structure; some related parameters are listed in Table 2.

Table 2. *Stereochemistry of the final models of* Co- *and* Ni-*substituted concanavalin A*: *r.m.s. deviations from ideality*

	Co-conA	Ni-conA
Bond lengths (Å)	0.012	0.013
Bond angles (°)	2.811	2.748
Dihedral angles (°)	26.090	26.049
Improper angles (°)	1.876	1.358

Table 3. *Average temperature factors* (Å^2)

(Resolution)	Co-conA (1.6 Å)	Ni-conA (2.0 Å)	Cd-conA (2.0 Å)	Nat. conA (2.0 Å)
Main chain	13.6	17.9	16.8	15.9
Side chain	20.2	21.8	21.5	21.5
Waters	30.9	36.7	35.0	39.5
TM	9.0	20.0	14.0	15.0
TM ligands	9.0	14.0	14.0	12.0
Ca	8.0	13.0	11.0	10.0
Ca ligands	9.0	13.0	13.0	12.0
All atoms	17.8	21.0	19.0	20.4

Note: TM = transition-metal ion.

86.5% of the non-glycine residues lie in the most favoured regions of the Ramachandran plot, none of these residues is found outside the allowed regions (Ramakrishnan & Ramachandran, 1965). The mean coordinate error given by a Luzzati plot lies in the range from 0.15 to 0.25 Å (Luzzati, 1952).

The model fits very well to the electron density. Only the main-chain atoms of the loop regions around residues 120 and 160 do not lie in 1 r.m.s. $2F_o - F_c$ density. Fig. 1 illustrates quite well that the electron density is better defined for the Co than for the Ni structure.

The highest peak in the $F_o - F_c$ map (5σ) is due to a second, partially occupied conformation of His51. Multiple side-chain conformations are observed for the side chains of the same residues as for the Co-substituted concanavalin A structure (see §2.5).

Average *B* values for the protein atoms and the water molecules are given in Table 3.

3. Results and comparisons

Most of the structural features of saccharide-free concanavalin A have been described in considerable detail elsewhere (Naismith *et al.*, 1993) and need not be discussed here. Instead we compare the structural results of the studies of Co- and Ni-substituted concanavalin A with the high-resolution studies of Cd-substituted (Naismith *et al.*, 1993) and native concanavalin A (Weisgerber & Helliwell, 1993).

The high similarity between these four metal derivatives becomes evident when the Co-substituted

concanavalin A structure is superimposed on the structures of the Ni-, Cd-substituted and native concanavalin A. The results of these and other superpositions are summarized in Table 4. All C^{α} atoms superimpose with r.m.s. deviations in the range 0.1–0.18 Å; the r.m.s. deviations for C^{α} atoms of the β-sheets only are even smaller (~0.07–0.13 Å). All deviations are less than the experimental error indicated by the Luzzati plot.

The major structural differences between the structures of Ni-, Co-, Cd-substituted and native concanavalin A are due to the presence of a third metal site at the dimer interface. Although the crystallization conditions are very similar for the single transition-metal crystals, only the Cd-substituted species has a third metal site. This gives rise to the assumption that in order to form a third metal-binding site the radius of the metal ion should be at least 1 Å. It is an intriguing question why Ca ions do not bind to this site *S*3 given that *S*2 binds both Ca and Cd ions and the chemical nature of the metal-binding ligands is the same in *S*3 as in *S*2.

Residues which are involved in binding the third metal ion are Glu87 and Glu183 as well as Asp136 of a symmetry-related subunit. The orientation of Glu87 is unaffected by the presence of a third metal ion whereas Glu183 and Asp136 adopt a different conformation in the structures with and without a third metal ion. In the structures without a third metal-binding site the orientation of Asp136 is stabilized *via* an intramolecular salt bridge between Asp136 and Lys138. Instead of binding to the Cd ion, Glu183 forms an intermolecular hydrogen bond to Gln132 of a symmetry-related subunit at the dimer interface (Table 5). As a consequence Gln132 adopts a slightly different conformation in the structures with and without a third metal site. The electron density around this metal site, which is located on the surface of the protein, is not as well defined as for other parts of the structure, nevertheless the presence of two water molecules in the Co as well as in the Ni structure, which do not appear in the Cd structure, is unambiguous. One of these water molecules (O*W*117 in the Co structure, O*W*71 in the Ni structure) seems to replace the carboxylate group of Gln132 in the Cd structure; the second one is found in place of the Oε2 atom of Glu183 (O*W*76 in the Co structure, O*W*102 in the Ni structure). Although there are quite a few different inter- and intramolecular hydrogen bonds around the third metal site (Table 5), the overall water network and the crystal packing do not otherwise change on binding a third metal ion.

In the structures of Co- and Ni-substituted concanavalin A the transition-metal site *S*1 is in an octahedral environment and the calcium-binding site *S*2 is in a distorted octahedral environment with both octahedra sharing a common ligand; the metals are 4.17 (0.01) Å apart (Table 6). The transition-metal ion is hexa-coordinate, five O atoms and one N atom forming the inner coordination sphere. Transition-metal to ligand distances and temperature factors of the ligands for the cobalt and the nickel structure are given in Table 6. The coordination sphere of the calcium ion consists of seven O atoms; Asp10 binds in a bidentate manner providing two O atoms (see Table 6 for Ca–ligand bond distances and ligand temperature factors).

A superposition of the metal-binding sites *S*1 and *S*2 of the four metal derivatives proves that the coordination sphere of the transition metal and the calcium ion is essentially identical, at the resolution of these structures. The orientation of the ligands directly involved in metal binding and the hydrogen-bonding pattern around the metals is unaffected by the nature of the transition metal. A comparison of

Table 4. *Superposition of the* Co-, Ni-, Cd-*substituted and native concanavalin A structures*

R.m.s. deviations (Å) for all Cα atoms. The corresponding values for the Cα atoms of the β-sheets only are in parentheses; the diagonal values are the upper values of estimate of error as indicated by the Luzzati plot.

	Co-conA	Ni-conA	Cd-conA	Nat. conA
Co-conA	0.20	0.15 (0.12)	0.11 (0.08)	0.10 (0.07)
Ni-conA		0.25	0.18 (0.13)	0.17 (0.13)
Cd-conA			0.25	0.10 (0.07)
Nat. conA				0.25

Table 5. *Protein–protein hydrogen-bond interactions which form the dimer in* Co- *and* Ni-*substituted concanavalin A*

Monomer 2 is generated from monomer 1 by applying the symmetry operation $(1 - x, 1 - y, z)$.

		Distance (Å)	
Monomer 1	Monomer 2	Co-conA	Ni-conA
Contacts which form the extended β-sheet			
*N Ala125	O Met129	2.91	2.82
*N His127	O His127	2.85	2.91
*N Met129	O Ala125	2.85	2.90
Remaining protein–protein hydrogen-bond interactions			
*Nε1 Trp88	O Asp136	3.43	3.44
*Nδ2 Asn124	O Gln132	2.89	2.82
*N Asn131	Oδ1 Asn124	2.75	2.80
*Nδ2 Asn131	O Thr123	2.83	2.84
*N Gln132	Oδ1 Asn124	3.34	3.32
Oγ Ser117	Oε1 Gln132	3.00	3.19
Oε1 Glu122	O Asn131	3.49	3.47
Oε2 Glu122	O Asn131	3.01	3.22
Oε1 Gln132	Oε1 Glu183	2.93	3.32
Oε1 Gln132	Oγ Ser185	3.35	3.33

* Interactions also found in Cd-substituted concanavalin A (Naismith *et al.*, 1993).

Table 6. *TM–ligand distances* (Å) *at the transition-metal site S1 and* Ca*–ligand distances* (Å) *at the* Ca *site S2 with temperature factors* (Å^2) *in brackets*

(*a*) TM–ligand distances

TM	Co-conA	Ni-conA	Cd-conA	Nat. conA
O*W A*	2.19 (10)	2.26 (12)	2.50 (16)	2.26 (14)
O*W B*	2.24 (8)	2.28 (14)	2.13 (13)	2.13 (9)
OD1 Asp10	2.12 (7)	2.13 (12)	2.14 (16)	2.13 (15)
OE2 Glu8	2.14 (8)	2.21 (16)	2.33 (16)	2.23 (14)
OD1 Asp19	2.19 (9)	2.17 (18)	2.40 (17)	2.26 (13)
NE2 His24	2.20 (10)	2.13 (10)	2.34 (13)	2.25 (9)
TM-*L* (av.)	2.18 (9)	2.20 (14)	2.31 (14)	2.21 (13)
TM-Ca	4.16	4.18	4.18	4.16

(*b*) Ca–ligand distances

Ca	Co-conA	Ni-conA	Cd-conA	Nat. conA
O*W C*	2.40 (10)	2.45 (12)	2.35 (13)	2.37 (13)
O*W D*	2.42 (7)	2.36 (15)	2.50 (11)	2.32 (10)
OD1 Asn14	2.37 (10)	2.57 (16)	2.60 (17)	2.55 (15)
OD2 Asp19	2.32 (10)	2.40 (13)	2.30 (12)	2.30 (11)
OD1 Asp10	2.47 (7)	2.51 (12)	2.52 (16)	2.45 (15)
OD2 Asp10	2.39 (9)	2.36 (13)	2.29 (13)	2.28 (10)
O Tyr12	2.32 (9)	2.39 (13)	2.32 (13)	2.31 (10)
Ca-*L* (av.)	2.38 (9)	2.43 (13)	2.41 (13)	2.37 (12)

Note: The water molecules are labelled for consistency with Naismith *et al.* (1993), Fig. 6 and Tables 7 and 8. *L* = ligand atom.

individual transition-metal to ligand bond distances (*i.e.* at the *S*1 site) shows that the corresponding values for the structures of native, Co- and Ni-substituted concanavalin A are similar; differences in the average distances are small, being within 0.03 Å (Table 6). The average Cd–ligand distance however, is about 0.1 Å longer than the corresponding value for the other derivatives because Cd has a substantially larger ionic radius than Ni or Co. There is also one large difference. Water molecule O*WA* moves away from the metal ion by 0.25 Å (Table 6). For site *S*2 the average calcium to ligand distances of the four structures are in very good agreement (Table 6), the differences for individual ligands are small.

The average temperature factors of the ligands are close to the values found for the transition metals and for the calcium (Table 3), indicating well ordered transition-metal- and calcium-binding sites. A comparison of the temperature factors of the transition metals and of the protein as a whole for each case (Table 3) shows increased order for the cobalt *versus* the nickel or cadmium forms. Kalb *et al.* (1988) postulated in a survey of the resolution limits of a variety of metal-substituted concanavalin A's, that there was increased disorder caused by the smaller transition-metal ions. For cobalt, nickel and cadmium, with oxidation state II and coordination number 6, the ionic radii are 0.89, 0.83 and 1.09 Å, respectively (Cotton & Wilkinson, 1988), where in the case of cobalt, high spin is assumed, because of the nature of the ligands. Hence, it is an oversimplification to correlate only with ionic radius. However, we have no explanation at present for the improved resolution limit for some metal ions over others.

Fig. 3 compares the sugar binding region in the four saccharide-free structures. The high degree of similarity around this site suggests that the errors of the structures are small and that comparisons of the structures of saccharide-free concanavalin A and saccharide complexes of this protein can be made with high reliability.

4. Concluding remarks

From our studies of Co- and Ni-substituted concanavalin A we can conclude that the overall geometry of the transition-metal-binding site *S*1 and the neighbouring calcium site *S*2 are essentially independent of the nature of the transition metal, at least at the resolution of these studies. Our studies indicate some correlation between the transition metal and the degree of disorder in the entire molecule and thus the resolution limit of the crystal. Multiple conformations of five amino-acid side chains, remote from the saccharide-binding site, have been clarified by virtue of the 1.6 Å resolution study of the Co-substituted concanavalin A structure. The coordinates of the Co- and Ni-substituted concanavalin A structures and the structure factors have been deposited with the Protein Data Bank.*

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* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1SCS, R1SCSSF for Co,Ca concanavalin A; 1SCR, R1SCRSF for Ni,Ca concanavalin A). Free copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Reference: LI0173).

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