THE TRANSITION METAL-BINDING SITE OF CONCANAVALIN A AT 2.8 Å RESOLUTION

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1. Introduction

Concanavalin A is a saccharide-binding protein of the Jack bean. Its molecular weight if 55,000 and it is composed of two identical subunits. The protein molecule contains two transition metal ion-binding sites. When these sites are occupied, two Ca^{2+}-binding sites are formed [1–4].

Concanavalin A crystals of space group I222 and unit cell dimensions $a = 87.2$ Å, $b = 89.2$ Å, and $c = 62.9$ Å have been studied by X-ray crystallographic methods. We have now computed a Fourier map of concanavalin A at 2.8 Å resolution by the multiple isomorphous replacement technique. A complete account of the methods used as well as a detailed interpretation of the entire map is in preparation. We wish now to present our interpretation of that portion of the map which is clearly identifiable as the transition metal-binding site.

2. Experimental

The crystals used in this study were made from demetallized concanavalin A [2]. They were grown in presence of 10^{-3} M $MnCl_2$ and 10^{-3} M $CaCl_2$ by dialysis against 0.1 M $NaNO_3$ with 0.05 M Tris-acetate, pH 6.5. The crystals were isomorphous with the native crystals previously described [4]. Isomorphous crystals of cadmium-concanavalin A were prepared as above except that 10^{-3} M $CdCl_2$ was used instead of $MnCl_2$. A single-site, isomorphous, heavy-atom derivative of manganese-concanavalin A was made by soaking the crystals in 10^{-3} M K_2PtCl_4 in the crystallization buffer.

The Fourier map was calculated on the basis of two derivatives, the $CdCl_2$ replacement and the K_2PtCl_4 substitution. The $PtCl_4^{2-}$ substitution resulted in a single Pt site which appeared elongated in a direction parallel to the z-axis. The Cd^{2+} replacement resulted in two major sites of substitution of approximately equal occupancy, and a third, minor site with coordinates close to those of the $PtCl_4^{2-}$ substitution. Anomalous scattering data were also collected for the $PtCl_4^{2-}$ derivative but have not yet been included in the phase analysis.

Approximate parameters for the two derivatives were refined by the method of least squares until the change in the residual from one cycle to the next was negligible. The $PtCl_4^{2-}$ temperature factor was treated as isotropic in this study due to limitations in the current refinement program. The final parameters used to calculate the Fourier map are given in table 1.

The map was visualized by tracing the positive electron density contours on clear plastic sheets representing sections parallel to the x-z plane and separated by 0.75 Å. The scale was 1 cm = 2 Å.

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Table 1

	x	y	z	Z	B	Erms	R
K_2PtCl_4	0.49706	0.44175	0.44389	3.000	50.26	4.33	0.089
$CdCl_2$, site 1	0.12898	0.15053	0.28220	1.149	13.15		
site 2	0.21180	0.30688	0.25505	1.136	21.41	2.77	0.061
site 3	0.49476	0.45350	0.45337	0.260	22.77		

The mean protein structure factor magnitude was 34.6. Erms = $<(|FPH_{obs}| - |FPH_{calc}|)^2>^{1/2}$. $R = <(|FPH_{obs}| - |FPH_{calc}|)/<|FPH_{obs}|>$. B is defined by the temperature factor expression: $t = \exp(-B \cdot \sin^2\theta/\lambda^2)$.

3. Results

In the region of the contour map $x = 0$–0.5, $y = 0$–0.5, $z = 0$–0.5 (one asymmetric unit), high electron density extends diagonally upwards from the origin and is bounded by regions of low electron density (fig. 1). The electron-rich region represents the general shape of the protein subunit and the low-density regions represent the intermolecular solvent. Four of these protein subunits are arranged about the origin in a tetrahedral cluster (as is required by space group symmetry) and appear to be paired off into two molecules, each consisting of two subunits in close contact and interlocked across the z-axis.

The manganese ion may be recognized in the map as the highest peak of electron density in the asymmetric unit. The coordinates of this peak correspond closely to the position of a major Cd^{2+}–Mn^{2+} substitution site (the other major Cd^+ site is in a region of low electron density) $x = 0.211$, $y = 0.305$, $z = 0.239$ (fig. 1). The transition metal is thus deeply embedded in the subunit and widely separated from transition metal sites in the symmetry-related subunits. The distance between the sites in a pair of subunits constituting a dimeric molecule of concanavalin A is 65.8 Å; between the sites in the two subunits related by the y-axis, 49.1 Å; and between the sites related by rotation about the x-axis, 63.5 Å.

A remarkable feature of the transition metal-binding site is its low symmetry. Two axially related ligands can be traced in the map. A third ligand is also apparent but appears much weaker in the electron density map than the other two. The remaining coordination positions appear to be in contact with two unconnected regions of low electron density, presumably solvent. The first of these regions does not appear to be connected to the inter-molecular solvent in the crystal and is roughly hemispherical in shape. The second region of low electron density is a continuous channel running from the transition metal position to the outside of the molecule. The channel is approximately 6 Å in cross sectional diameter and 15 Å long and runs nearly parallel to the z axis. Thus, the transition metal, although deep in the interior of the subunit, is indirectly in contact with the solvent which bathes the molecule.

In aqueous solution, the transition metal-binding site of concanavalin A binds ions of widely different size such as Mn^{2+} and Cd^{2+}. The properties of the Ca^{2+}-binding site and of the saccharide-binding site are, however, insensitive to the size of the transition metal ion [5]. This would suggest that the transition metal site can accommodate various transition metals without necessitating large movements of protein. The present finding that Cd^{2+}-concanavalin A is isomorphous with Mn^{2+}-concanavalin A confirms this. In a recent study

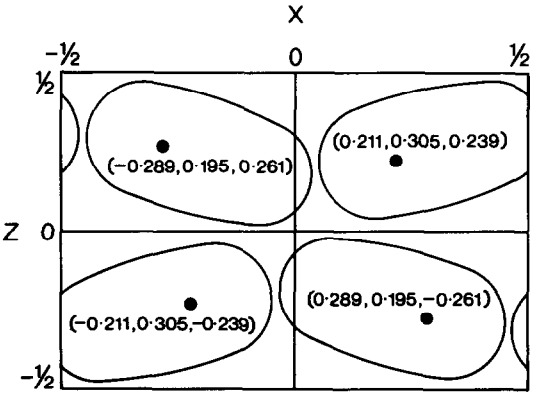

Fig. 1. Diagram of the x-z projection of the unit cell showing two molecules of concanavalin A. The transition metal sites are indicated by darkened circles. The metal site coordinates are in fractional coordinates.

of crystals of demetallized concanavalin A, no large intensity changes were observed in the diffraction pattern when the crystals were allowed to bind Mn^{2+} [6]. This again suggests that only subtle movements of protein occur on binding transition metal ions. The transition metal-binding site as seen at 2.8 Å resolution represents a flexible metal-protein configuration. The absence of protein ligands on one side of the metal ion and the roomy solvent dome on that side would tend to localize most of the structural changes associated with the binding of a transition metal within the dome itself, perhaps by rearrangement of the solvent.

Electron spin resonance studies of concanavalin A in solution [7] and in single crystals [8] have indicated that the coordination symmetry of the transition metal site is low. Axial symmetry can be assigned on the basis of the 2.8 Å map if the weaker third ligand is assumed to be loosely bound and if the axial ligands are identical or closely similar. The symmetry could, of course, be even lower than axial.

Release of transition metal ions from concanavalin A is very slow [7]. Entrapment of the transition metal deep within the interior of the protein may hinder its release. The solvent channel would provide a likely pathway for an escaping metal ion, but its limited dimensions might restrict the movement of the ion. Restrictions should also apply to diffusion of a metal ion into the site. A study of kinetics of metal ion-binding in concanavalin A should help to clarify this.

The solvent channel should provide a pathway for solutes of small size to approach the transition metal in the binding site. In particular, water molecules from the outside might be expected to exchange with water molecules in direct contact with the transition metal ion at a rate which would be limited by the transport rate through the channel. Preliminary measurements of proton paramagnetic relaxation in aqueous solution of Mn^{2+}-concanavalin A indicate that Mn^{2+} in the site does have access to the bulk solvent but that access is not unrestricted [8].

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