The Coordination Polyhedron of Ca^{2+}, Cd^{2+} in Parvalbumin

AMY L. SWAIN* and E. L. AMMA**

Department of Chemistry, University of South Carolina, Columbia, SC 29208 (U.S.A.)

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The calcium ion is ubiquitious in the biological scheme and, therefore, it is no surprise that the bioinorganic chemistry of Ca^{2+} is an active and burgeoning research area. Parvalbumins are small globular calcium-binding proteins which have long been a reference point for a class of proteins in which the calcium ion is associated with a helix–loop–helix motif of calcium binding [1, 2]. Members of this class are troponin C, calmodulin, intestinal calcium-binding protein and others [2, 3]. This helix–loop–helix calcium-binding domain of parvalbumin was first reported by Kretsinger *et al.* and was termed as CD or EF hand sites to distinguish the two independent sites [4–6], and they were described as six-coordinate and eight-coordinate respectively [5].

The ^{113}Cd NMR spectra of these proteins [7–10] are unique in that they are all very shielded (≈−100 ppm) with respect to the $Cd(ClO_4)_2 \cdot 6H_2O$ standard. It has been proposed from model studies that these shielded values are due to coordination numbers higher than six [11]. The discrepancy in coordination number based on the ^{113}Cd NMR studies and the crystallographic results prompted this investigation on: (i) the suitability of ^{113}Cd as a probe for calcium binding sites and (ii) a re-examination of a long accepted model for EF hand proteins.

Experimental

The carp muscle calcium-binding parvalbumin crystals were provided by Prof. R. H. Kretsinger. The cadmium-substituted protein was obtained from Dr Paul Marchetti [7]. Protein purity was determined [1] and cadmium content was confirmed [12]. Crystals were grown by the sitting drop vapor diffusion technique at 18 °C [13]. The crystals were mounted by standard techniques. X-ray intensity data were collected for both crystals at MAXD [14]. The calcium parvalbumin data were collected at 4 °C and the Cd-substituted parvalbumin data at 18 °C. Crystal orientation, data collection and data reduction were carried out with facility programs.

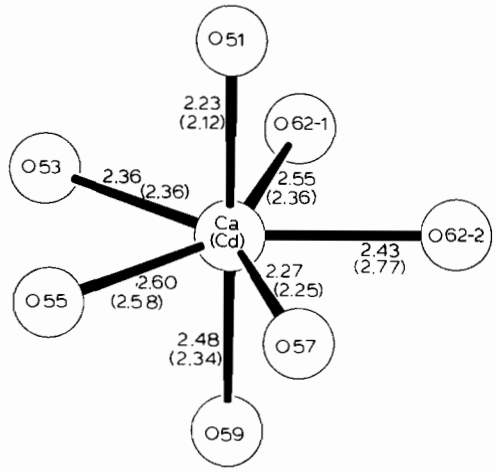

Fig. 1. An idealized pentagonal bipyramid with the observed distances for the CD site of the metal atoms in parvalbumin. The Cd distances are in parentheses. The observed angles are given in Table 1. The pentagonal plane is defined by atoms: O53 (carboxyl oxygen of Asp53), O55 (Ser OH), O57 (Phe, backbone C=O oxygen), O62-1, O62-2 (chelating carboxyl oxygens of Glu62). The axial positions are made up from: O51 (carboxyl oxygen of Asp51), O59 (carboxyl oxygen of Glu59).

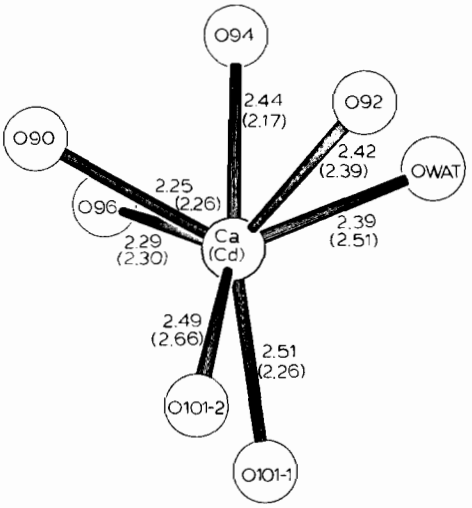

Fig. 2. An idealized monocapped trigonal prism with observed distances for the EF site of the metal atoms in parvalbumin. The Cd distances are in parentheses. The observed interatomic angles are given in Table 1. The capping atom is O94 (carboxyl oxygen from Asp94). A trigonal face is defined by: O90 (carboxyl oxygen of Asp90), O92 (carboxyl oxygen of Asp92), O101-2 (one carboxyl oxygen of Glu101). The other trigonal face is defined by: O97 (oxygen of backbone carbonyl of Lys96), OWATER, O101-1 (other carboxyl oxygen of Glu101).

*Present address: NCI-Frederick Cancer Research Facility, P.O. Box B, Frederick MD 21701, U.S.A.

**Author to whom correspondence should be addressed.

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TABLE 1. O–Ca–O (O–Cd–O) angles (°)

CD site pentagonal bipyramid:			
O51–Ca–O53	85.6 (81.3)	O53–Ca–O55	81.1 (77.5)
O51–Ca–O55	86.4 (89.4)	O53–Ca–O57	152.4 (147.2)
O51–Ca–O57	80.9 (82.8)	O53–Ca–O59	101.6 (106.2)
O51–Ca–O59	168.8 (169.2)	O53–Ca–O62-1	72.5 (75.2)
O51–Ca–O62-1	102.0 (99.0)	O53–Ca–O62-2	123.9 (125.1)
O51–Ca–O62-2	102.8 (99.7)		
O55–Ca–O57	74.2 (74.0)	O57–Ca–O59	89.0 (86.9)
O55–Ca–O59	86.2 (84.7)	O57–Ca–O62-1	133.8 (135.7)
O55–Ca–O62-1	151.4 (149.8)	O57–Ca–O62-2	82.7 (85.7)
O55–Ca–O62-2	153.6 (156.6)	O59–Ca–O62-1	88.5 (90.7)
O62-1–Ca–O62-2	51.4 (50.2)	O59–Ca–O62-2	80.6 (82.7)
EF site, monocapped trigonal prism:			
O90–Ca–O92	82.2 (77.7)	O92–Ca–O94	79.6 (80.4)
O90–Ca–O94	86.7 (86.5)	O92–Ca–O96	156.0 (155.4)
O90–Ca–O96	87.8 (90.7)	O92–Ca–OWAT	94.6 (102.1)
O90–Ca–OWAT	162.6 (165.7)	O92–Ca–O101-1	124.4 (120.3)
O90–Ca–O101-1	116.4 (113.6)	O92–Ca–O101-2	80.0 (74.4)
O90–Ca–O101-2	90.4 (86.9)	O94–Ca–O96	78.1 (77.2)
O96–Ca–OWAT	88.5 (83.9)	O94–Ca–OWAT	76.0 (79.4)
O96–Ca–O101-1	79.7 (84.2)	O94–Ca–O101-1	147.1 (152.8)
O96–Ca–O101-2	122.0 (127.1)	O94–Ca–O101-2	159.6 (154.8)
OWAT–Ca–O101-1	79.5 (79.1)	O101–1Ca–O101-2	50.2 (49.8)
OWAT–Ca–O101-2	105.9 (106.9)		

Crystal data:	a (Å)	b (Å)	c (Å)	β (°)	NF	R_f
Ca-parvalbumin	28.70	60.61	54.35	94.6	10 000	0.165
Cd-parvalbumin	28.44	60.46	54.12	94.8	8863	0.187

(NF = number of non-zero reflections, based upon $I > 3\sigma$).

Approximately 75 water molecules were included in the final refinement; 190 cycles were run for Ca and 160 for the Cd protein. Space group = $C2$; $R_f = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ where subscript f refers to final value. Function minimized = $\Sigma w_1(|F_o| - |F_c|)^2 + \Sigma w_2(s^{ideal} - s^{model})^2$, in which s incorporates all the stereochemical features such as bond distances, angles, etc. All of the RMS δ values were equal to or less than the target values, e.g., distance target values were 0.02 Å. Crystal size: Ca-parvalbumin 0.45 × 0.25 × 0.1 mm; Cd-parvalbumin 0.8 × 0.15 × 0.15 mm.

Three software packages were used in the refinement that started from atomic coordinates provided by the Protein Data Bank [15]. These packages were: PROTEIN [16], PROLSQ [17] and FRODO [18] on the VAX-11/780 and the E & S PS330. PROLSQ restrains interatomic distances, angles, etc. of the protein to accepted values. However, no restraints were placed upon the coordinates of the metal and it was free to define its coordination sphere. Details of the data collection and refinement, as well as protein structure details, will be reported elsewhere. The final conventional R was 0.187 for parvalbumin and 0.164 for the Cd derivative. The error in atomic positions was estimated [19] to be between 0.10 Å and 0.15 Å, a low value compared to most protein refinements. However, this value is at least an order of magnitude higher than that from small molecule refinements. The final atomic coordinates and X-ray intensity data will be submitted to the Protein Data Bank. We confine our discussion here only to the metal atom coordination polyhedra.

Results and Discussion

Of considerable importance to the utility of ^{113}Cd NMR as a Ca probe is the observation that the Ca and Cd sites in parvalbumin are essentially the same. However, the refined structures considerably change the interpretation of the coordination geometry of the metal atom. The CD site should be more correctly described as a seven-coordinate pentagonal bipyramid geometry rather than six-coordinate (Fig. 1, Table 1). This is now consistent with the refinement of other EF hand proteins [20] and other calcium-binding proteins [21, 22]. This coordination geometry has been observed in a number of model compounds with Cd, and it was postulated that this higher coordination number was the reason for the very shielded values found for the ^{113}Cd NMR in parvalbumin and related proteins [23, 24]. The EF site is now described as a seven-coordinate side mono-capped trigonal prism. (See Fig. 2 for details.) This particular geometry has not been previously observed for either Ca or Cd in proteins. This result may be a demonstration of the expected small energy differences between these two geometries for coordination number seven [25].

Nevertheless, these geometrical differences between the CD and EF sites are sufficient to understand the ^{113}Cd NMR isotropic chemical shift difference of 3.7 ppm (−97.5, −93.8 ppm) [8]. Although many problems, such as the insensitivity of the isotropic chemical shift, continue to exist in the unambiguous assignment of a particular ^{113}Cd NMR resonance to a particular coordination geometry and donor ligands, it seems clear at this time that such shielded values as ≈−100 ppm belong to higher coordination (greater than 6) in an all oxygen environment. It remains ambiguous which site corresponds to which resonance. This interpretation excludes the presence of 'ligands' such as nitrate and sulfate which should be very shielding and are not very relevant in protein systems.

One disturbing feature of the refinement is that in each site there appears to be a short Cd–O distance. In the CD site the Cd–O51 distance is 2.12 Å and in the EF site the Cd–O94 distance is 2.17 Å. Although these distances are within the quoted errors, the general consistency between the Ca and Cd structures is much better than these values would indicate. The shortest expected Cd–O distance should be 2.26 Å [26]. The other discrepancies between the Ca and Cd structures involved the chelating oxygen atoms. The Cd–O distances are much more asymmetric than the equivalent Ca–O distances. This is to be expected from the results of small molecule studies. Nevertheless, we feel it is safe to say at the present state of the art of protein refinement that the Ca and Cd sites are sufficiently similar to justify the continued use of Cd as a probe of Ca sites in proteins.

Supplementary Material

Final atomic coordinates and X-ray intensity data are available from the authors upon request.

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