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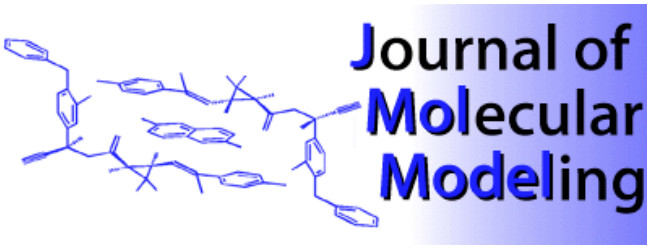

FULL PAPER

Zinc's Exclusive Tetrahedral Coordination Governed by Its Electronic Structure

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Abstract Zinc is a critical component of more than 300 proteins including farnesyltransferase, matrix metalloproteinases and endostatin that are involved in the front-line cancer research, and a host of proteins termed zinc fingers that mediate protein-protein and protein-nucleic acid interactions. Despite the growing appreciation of zinc in modern biology, the knowledge of zinc's coordination nature in proteins remains controversial. It is typically assumed that Zn^{2+} coordinates to four to six ligands, which led to intensive debates about whether the catalysis of some zinc proteins is regulated by zinc's four- or five-coordinate complex. Here we report the inherent uncertainty, due to the experimental resolution, in classifying zinc's five- and six-coordinate complexes in protein crystal structures, and put forward a tetrahedral coordination concept that Zn^{2+} coordinates to only four ligands mainly because of its electronic structure that accommodates four pairs of electrons in its vacant $4s4p^3$ orbitals. Experimental observations of five- and six-coordinate complexes were due to one or two pairs of ambidentate coordinates that exchanged over time and were averaged as bidentate coordinates. This concept advances understanding of zinc's coordination nature in proteins and the means to study zinc proteins to unlock the secrets of Zn^{2+} in human biology. In particular, according to this concept, it is questionable to study zinc's coordination in proteins with Co^{2+} as a surrogate of Zn^{2+} for spectroscopic measurements, since the former is a d^7 unclosed shell divalent cation whereas the latter is a d^{10} closed shell divalent cation.

Keywords Zinc proteins, Endostatin, Farnesyltransferase, Matrix metalloproteinases, Zinc finger

Running Title Exclusive Tetrahedral Coordination of Zinc

Introduction

About one third of proteins are known to require a metal ion for their structure and function [1]. Following iron, zinc is the second most abundant transition metal in biology [2]. An average adult human contains about 2.3 g of zinc com-

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pared to 4.0 g of iron [2]. Zinc is a critical component of more than 300 proteins [3,4] including farnesyltransferase (FT) [5], matrix metalloproteinases [6-8] and endostatin [4] that are involved in the front-line cancer research, and a host of proteins termed zinc fingers that mediate protein-protein and protein-nucleic acid interactions [9]. In addition, zinc is involved in the dimerization of human growth hormone and increases the affinity of human growth hormone for the prolactin receptor. It also inhibits the biological activities of nerve growth factor and related neurotrophins by blocking to their receptors.

Despite the growing appreciation of zinc in modern biology, the knowledge of zinc's coordination nature in proteins remains controversial. It is typically assumed that Zn^{2+} coordinates to four to six ligands [10], which has led to intensive debates about whether the catalysis of some zinc proteins is regulated by zinc's four- or five-coordinate complex [11]. Our interest in zinc stemmed from its presence in FT [5], an enzyme that modifies pro-*Ras* mutants responsible for cancer cell proliferation [12-14]. To develop effective FT inhibitors useful in blocking cancer cell proliferation, we endeavored to perform molecular dynamics (MD) simulations of FT [15] in water with a nonbonded zinc model (i.e., the zinc ion is not covalently bonded to its ligands) [16] employing the AMBER5.0 program [17]. With our nonbonded parameters for the zinc ion developed from a model in which

Figure 1 *Ideal and Observed Zinc Complexes*

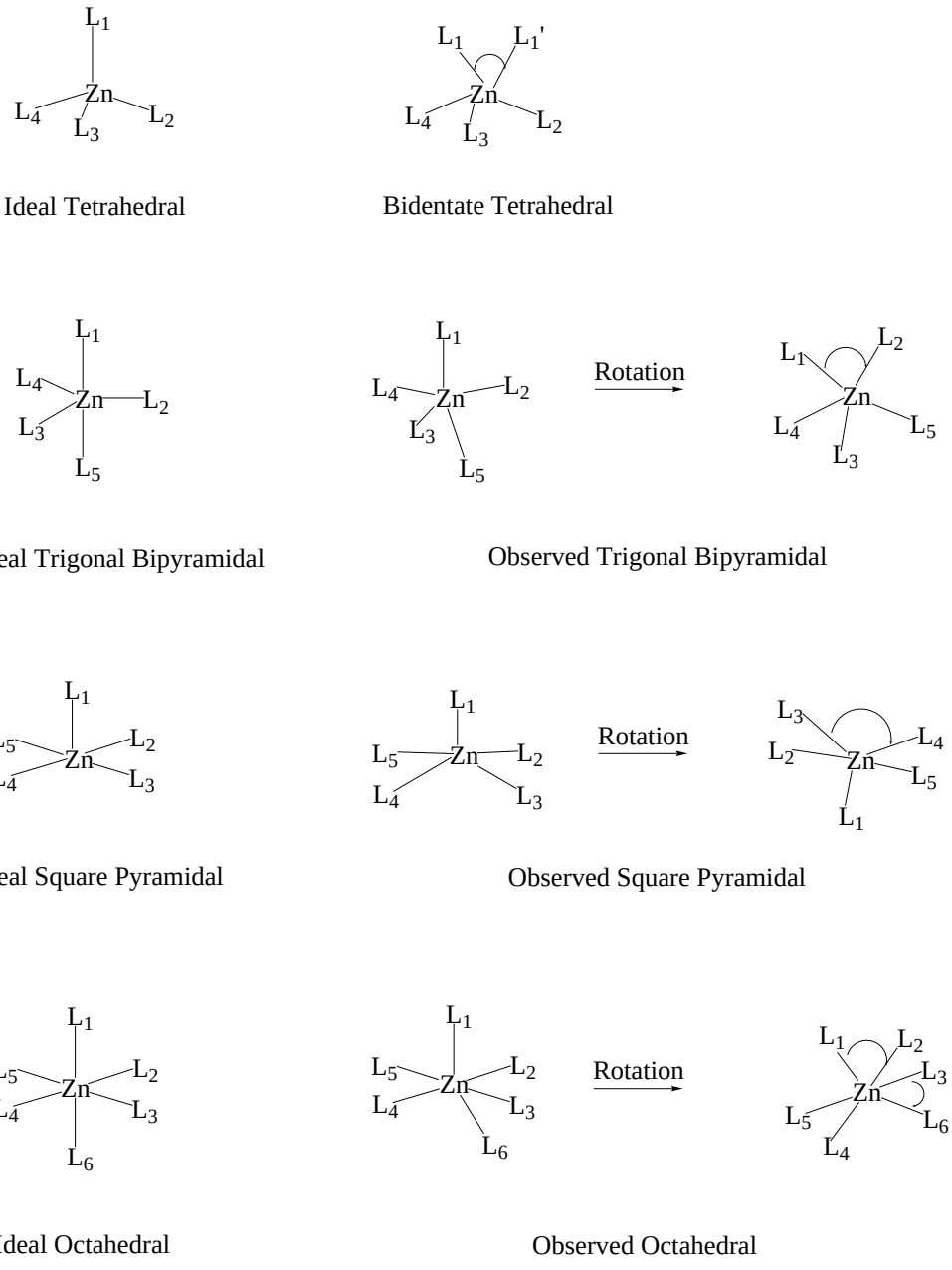

Table 1 *The 62 selected zinc proteins*

PDB Code[a]	Res. (Å)	Zinc's B-Value	Geometry[b]	PDB Code[a]	Res. (Å)	Zinc's B-Value	Geometry[b]
1IRN	1.20	9.99	T	1SLM	1.90	19.97	T
1PPT	1.37	NA	T			21.24	T
1XSO	1.49	9.34	T	1AZV	1.90	26.93	T
		8.92	T			20.97	T
1EZM	1.50	11.91	T	1CLC	1.90	28.38	T
1AH7	1.50	2.00	P (86°)	2NLL	1.90	56.36	T
		2.00	P (82°)			44.58	T
		2.00	TB (78°)			17.49	T
2CTB	1.50	7.34	TB (56°)			23.79	T
2CBA	1.54	7.76	T	4ENL	1.90	13.48	TB (80°)
1HFC	1.56	6.28	TB (76°)	1FUA	1.92	12.51	TB (60°)
		7.64	T	1PTQ	1.95	16.16	T
1AAY	1.60	31.65	T			13.25	T
		23.91	T	1ZIO	1.96	14.50	T
		19.72	T	1F3Z	1.98	37.65	T
1BTK	1.60	19.19	T	1ALK	2.00	15.30	T
		13.20	T			14.98	TB (56°)
2MYR	1.60	9.19	T			12.89	TB (57°)
1KUH	1.60	1.63	T			12.84	TB (73°)
1ZIN	1.60	17.10	T	1STE	2.00	11.71	T
1LAM	1.60	21.83	TB (73°)	1BRH	2.00	10.49	T
		9.30	T	1TAF	2.00	18.18	T
		9.71	TrB (84°)			14.94	T
8TLN	1.60	16.45	T			22.82	T
1KAP	1.64	11.74	T			20.09	T
3BTO	1.66	11.80	T			35.81	TB (73°)
		12.24	T	1FRP	2.00	49.79	TB (58°)
		11.28	T			50.20	TB (58°)
		10.67	T	1XER	2.00	11.98	T
		9.60	T	2EBN	2.00	43.18	T
		12.26	T	4MT2	2.00	18.57	T
		10.21	T			22.65	T
		10.04	T	1IAG	2.00	20.62	T
1VHH	1.70	7.53	T	1RMD	2.10	34.77	T
1PMI	1.70	12.92	P (82°)			23.28	T
1HML	1.70	19.80	T			24.14	T
1SAT	1.75	10.68	T			23.33	T
1XJO	1.75	13.82	T	1CFV	2.10	39.38	T
		11.27	TB (60°)			18.98	T
1ENR	1.80	11.32	O (83°, 87°)			18.70	T
1TON	1.80	6.10	T	1AUI	2.10	63.83	T
2TCI	1.80	12.88	T	1DPM	2.10	18.56	TB (79°)
		16.33	T			21.41	T
8RNT	1.80	21.89	O (76°, 81°)			13.95	TrB (84°)
1ATL	1.80	14.23	TB (61°)			18.32	T
		15.55	TB (60°)	1CTT	2.20	22.40	T

[a] References for protein structures are available in the PDB coordinate files.

[b] T: tetrahedral; TB: tetrahedral with bidentate ligands; TrB: trigonal bipyramidal; P: square pyramidal; and O: octahedral; Angles of L1-Zn-L1', L1-Zn-L2, L3-Zn-L4 and L3-Zn-L6 respectively are listed in parentheses. Distances and angles were calculated by using the Quanta 97 program [25].

Table 1 (cont.) *The 62 selected zinc proteins*

PDB Code[a]	Res. (Å)	Zinc's B-Value	Geometry[b]	PDB Code[a]	Res. (Å)	Zinc's B-Value	Geometry[b]
1AST	1.80	10.40	T	1LBA	2.20	22.91	T
1JAP	1.82	16.64	TB (79°)	1FRO	2.20	19.06	P (83°)
		13.66	T			19.06	P (83°)
1PUD	1.85	12.61	T			19.06	P (83°)
1BME	1.85	26.08	T			19.06	P (83°)
		23.76	T	1TSR	2.20	27.14	T
1HXQ	1.86	18.93	T			28.10	T
		20.38	T			41.30	T
1LML	1.86	16.09	T	1FT1	2.25	27.46	T
1MMQ	1.90	17.71	T	1JAQ	2.25	10.57	T
		14.51	TB (80°)			10.04	T

[a] References for protein structures are available in the PDB coordinate files.
[b] T: tetrahedral; TB: tetrahedral with bidentate ligands; TrB: trigonal bipyramidal; P: square pyramidal; and O: octahedral; Angles of L1-Zn-L1', L1-Zn-L2, L3-Zn-L4 and L3-Zn-L6 respectively are listed in parentheses. Distances and angles were calculated by using the Quanta 97 program [25].

the zinc ion is coordinated with six water molecules in an ideal octahedral geometry according to the literature protocols [16,18], we found that the tetrahedral zinc complex, coordinated to $Cys^{299\beta}$, $His^{362\beta}$, $Asp^{297\beta}$ and H_2O^{1002} in FT, was immediately changed to a trigonal bipyramidal complex in which the zinc ion coordinates to $Asp^{352\beta}$ as well (unpublished result). The five-coordinate complex occurred regardless of whether the long-range electrostatic interactions were calculated in the MD simulations [19]. This observation raised two fundamental questions: 1) Does Zn^{2+}, as a d^{10} closed shell divalent cation, really form a five- or six-coordinate complex [10]? 2) Are the nonbonded zinc parameters developed from a six-coordinate complex in water applicable to simulations of a four-coordinate complex in proteins? These questions prompted us to investigate the zinc coordination patterns and the bonding nature of the zinc complexes in the protein crystal structures documented in the June 1998 release of the Protein Data Bank (PDB) [20].

Experimental procedures

Using the PDB 3DB Browser provided by the PDB world wide web site at the Brookhaven National Lab of the US in 1998, we found 407 crystal structures of zinc proteins documented in the PDB on June 5, 1998. These proteins contain a single or multiple zinc binding sites that play either a functional or structural role. We did not segregate the proteins with catalytic zinc binding sites from the ones with structural zinc binding sites, since our objective was to study general coordination patterns and bonding nature of the zinc complexes. Instead, we selected structures with resolutions higher than or equal to that of FT (2.25 Å) at which resolution the side chain structures of the proteins are defined by the electron density map [15]. To avoid bias due to certain proteins such as insulin, which has been extensively studied and has 23 structures documented in the PDB, we used one structure that has the highest resolution and discaarded the other structures of the same protein. We did not use structures with an irregular zinc complex and in which the zinc ions were used to improve the quality of the crystals (e.g., verotoxin, PDB code: 1BOY). These considerations led us to select the 62 structures listed in Table 1. In the structure of the DTAFII42-DTAFII62 complex (PDB code: 1TAF), we did not include the two irregular zinc complexes (Zn^{2006} and Zn^{2007}) in which the zinc ions have a much higher B-factor than the average B-factor of the protein. Altogether, we selected 114 zinc atoms and 489 associated zinc coordinates in the 62 zinc proteins.

For the coordinate distances, we rounded off the second digit after the decimal, since the average distances were based on proteins with resolutions varying from 1.20 to 2.25 Å. To examine if our selection of 62 structures is sufficient, we calculated the average coordinate distance per specific residues (Table 2) in order to examine the difference between the Zn-O distances from Glu and Asp. In principle, the Zn-O distances of Glu and Asp should be the same, since both contain a carboxylate group and are populated significantly and almost equally (Table 2). A discrepancy between the two average distances would reflect that the number of structures studied is insufficient. Our calculated average Zn-O distances from Asp and Glu are 2.1 ± 0.2 Å (54) and 2.1 ± 0.2 Å (40), respectively. This indicates that the number of structures used in the present study is sufficient.

Results and discussion

Examining the 114 zinc complexes in the 62 proteins, we first found that none of the five-coordinate complexes could be qualified as an ideal square pyramidal or trigonal bipyramidal complex. Nor could the six-coordinate complexes be identified as an ideal octahedral complex. Instead, the observed, distorted five-coordinate complexes were something between a tetrahedral complex with a bidentate coordinate (”bidentate” tetrahedral complex, Figure 1) and an ideal square pyramidal or trigonal bipyramidal complex (Figure 1). This observation is consistent with the report that the average angle of L1-Zn-L5 of the five-coordinate complexes (Figure 1) ranges from 154±8 (13) to 157±18 (6) degrees of arc and that the average angle of L1-Zn-L6 the six-coordinate complexes (Figure 1) in the octahedral complex ranges from 152±19 to 161±12 degrees of arc, rather than being 180 degrees of arc in ideal square pyramid, trigonal bipyramid and octahedron [21]. As depicted in Figure 1, rotating the observed, distorted trigonal bipyramidal complex to the perspective of the bidentate tetrahedral complex, it is conceivable that the main difference between the trigonal bipyramidal and the bidentate tetrahedral complexes is the angle difference between L1-Zn-L1' of the bidentate tetrahedron and L1-Zn-L2 of the trigonal bipyramid (Figure 1). If the two angles are identical, the two complexes are then interchangeable. To distinguish the bidentate tetrahedral complex from the pyramidal and bipyramidal complexes, we used the reported cutoff of 80 degrees of arc for angle L1-Zn-L1' (L1-Zn-L2), which is 90 degrees of arc in an ideal pyramidal or bipyramidal complex [21]. If the calculated L1-Zn-L1' (L1-Zn-L2) is equal to or less than the cutoff, the complex is considered as a bidentate tetrahedron. Otherwise, it is either a square pyramid or trigonal bipyramid. Accordingly, we found that the percentages of the zinc ions that form a tetrahedral, bidentate tetrahedral, square pyramidal, trigonal bipyramidal and octahedral complexes are 74%, 16%, 6%, 2% and 2%, respectively.

On the basis of the above findings, we reason that the zinc-complex geometry is mainly determined by the four vacant orbitals of the zinc divalent cation ($4s4p^3$), that energetically favorably accommodate four pairs of electrons from the zinc coordinates in proteins, and not by repulsion among the coordinated electron pairs of the coordinates [21]. First, the zinc complex is more stable when its principal quantum shell ($4s4p^3$) is filled according to basic quantum mechanics.

Table 2 *Average Coordinate Distances with Standard Deviations per Specific Residue Ligand*

Residue (Zinc Element)	Distance (Å)	Number of ligands
His (N)	2.1 ± 0.1	169
Cys (S)	2.3 ± 0.1	115
H_2O (O)	2.2 ± 0.2	60
Asp (O)	2.1 ± 0.2	54
Glu (O)	2.1 ± 0.2	40
Protein Ligand (O)	2.2 ± 0.3	17
Peptide (O)	2.4 ± 0.3	12
PO_4	2.1 ± 0.1	7
Gln (O)	2.0 ± 0.1	5
Lys (N)	2.1 ± 0.2	3
Terminal Peptide (N)	2.1 ± 0.1	2
Ser (O)	2.1 ± 0.02	2
Asn (O)	2.0 ± 0.1	2
Protein Ligand (N)	1.7	1

Second, in the structure of 2EBN (Table 1), the tetrahedral zinc complex is composed of H_2O (2.840 Å) and side chains of His (2.244 Å), Glu (2.210 Å) and Glu (2.580 Å). If the coordination pattern was governed mainly by the repulsion among the coordinated electron pairs of the coordinates, one would not observe the five-coordinate complexes in the structure of 1FRO (Table 1), since these complexes are composed of the same coordinates in the tetrahedral complex in 2EBN plus Gln side chain, namely His (2.024 Å), Glu (2.019 Å), Glu (1.994 Å), Water (2.111 Å), and Gln (2.016 Å). According to the two sets of zinc-coordinate distances listed in parentheses, zinc has tighter binding coordinates in the five-coordination in 1FRO than in the four-ligation in 2EBN, which contradicts the literature assumption that the zinc-complex geometry is mainly determined by repulsion among the coordinated electron pairs of the coordinates [21]. More examples to disprove the literature assumption can be found in protein pairs of 1AH7 with 1KUH, 2CTB with 1F3Z, 1VHH with 1AH7, 1STE with 1AH7, 2CTB with 2EBN, 2CTB with 1CFV, 1PMI with 1F3Z, 1ENR with 1TAF and 4ENL with 1TAF.

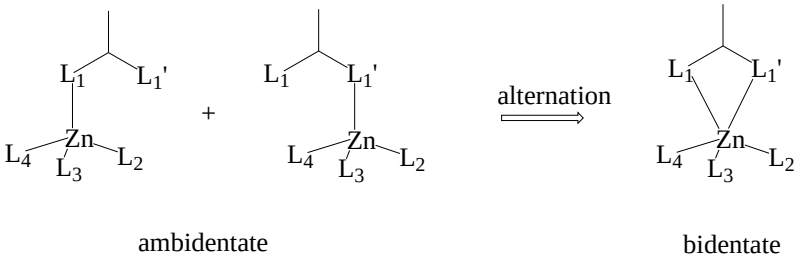

Figure 2 *Formation of the bidentate Tetrahedral Zinc Coordination*

Table 3 *Average Ligand Distances with Standard Deviations (Å): Coordination Pattern versus Type of Ligand (Number of ligands are given in parentheses)*

Coordination	Zn-N	Zn-O	Zn-S	Zn-X
4	2.1 ± 0.2 (162)	2.2 ± 0.2 (155)	2.3 ± 0.1 (115)	2.2 ± 0.2 (432)
5	2.1 ± 0.1 (12)	2.1 ± 0.1 (33)	0	2.1 ± 0.1 (45)
6	2.1 (1)	2.3 ± 0.1 (11)	0	2.3 ± 0.1 (12)
Total	2.1 ± 0.1 (175)	2.2 ± 0.2 (199)	2.3 ± 0.1 (115)	2.2 ± 0.2 (489)

Furthermore, we found that the difference between the L1-Zn-L2 angle of the identified trigonal bipyramid and the angle cutoff (80 degrees of arc) to qualify as the bidentate tetrahedral complex is only up to 7 degrees of arc, so are the differences in the cases of square pyramid and octahedron. However, given the average zinc coordinate distance of 2.2 Å (Table 3), a displacement of 0.3 Å of a zinc coordinate atom because of the imprecision in the atomic coordinates results in 8 degrees of arc uncertainty in the zinc coordinate angle. The uncertainty is estimated by $\Delta\alpha = \text{atan}(\Delta D/D)$, where ΔD is 0.3 Å which is obtained from the maximal standard deviation of the calculated zinc coordinate distances (Tables 2 and 3), and D is the average zinc coordinate distance. It is, therefore, uncertain if these complexes can be qualified as pyramidal, bipyramidal or octahedral complexes. For example, in the structure of homodimeric phospho-triesterase (PDB cod: 1DPM), one zinc complex is a bidentate tetrahedron and the other is a trigonal bipyramid (Table 1). The discrepancy in coordination pattern between the two identical proteins just revealed the inherent uncertainty, due to the experimental resolutions, in classifying zinc's polyhedral complexes.

In contrast to the above findings, Zn^{2+} is conventionally assumed to coordinate to four to six ligands [10]. The experimentally observed, distorted five- or six-coordinate complexes are accordingly classified as five- or six-coordinate complexes which uses more energetic $4s4p_x4p_y4p_z5s(4d)$ orbitals than the four-coordinate complex. This classification has caused intensive debates about whether the catalysis of some zinc proteins is regulated by a four- or five-coordinate zinc complex [11].

In light of the experimental resolutions and the above-mentioned determining factor of the zinc-complex geometry, we think that the five- and six-coordinate complexes in proteins should conceptually be viewed as tetrahedral complexes with one or two pairs of *alternate* coordinates. When the zinc divalent cation encounters an extra number of coordinates, alternation occurs, namely, one of the $4s4p^3$ hybrid orbitals of zinc alternately accommodates two coordinates that appear as bidentate coordinates in the protein crystal structures (Figure 2). In the case of six-coordinate complexes, two of such orbitals alternately accommodate four ligands. The alternation theory is consistent with our MD simulations of carboxypeptidase A in which the two oxygen atoms of the carboxylate group of Glu72 coordinate to Zn^{2+} in the structure averaged over a 2.0 ns MD simulation, but only one oxygen atom coordinates to the zinc ion in all the snapshots taken at 1.0 ps intervals (unpublished results). We further refer to the *alternate* coordinates as *bidentate coordinates* in a relatively long time frame (>2.0 ns) in which the alternation is averaged, and as *ambidentate coordinates* in a relatively short time frame (<2.0 ps) in which the alternation is not averaged.

As apparent in Table 3, the distances in "different" coordination patterns are the same, indicating that the ligands in "different" coordination patterns have the same bonding nature, namely, these coordinates occupy the same $4s4p^3$ orbital of zinc. The exclusive four-coordinate nature of the zinc divalent cation in proteins is further supported by theoretical studies of the zinc divalent cation complexed with water molecules in the gas phase using Density Functional Theory (DFT) [22,23]. The DFT calculations revealed that *in vacuo* the six-water zinc complex, is less stable by 5.4 kcal/mol than the four-water zinc complex in which the water molecules that are directly coordinated to zinc also interact with two water molecules that are not in the coordination shell [22]. Similarly, *in vacuo* the five-water zinc complex is less stable by 1.6 kcal/mol than the four-water zinc complex interacting with one water molecule that is not in the coordination shell [22]. However, the five-water zinc complex is more stable by 12 kcal/mol *in vacuo* than the three-water zinc complex interacting with two water molecules that are not in the coordination shell [22].

Distinguishing the bidentate tetrahedral complexes from the trigonal bipyramidal, square pyramidal and octahedral complexes seems semantic. *The essence is that the zinc divalent cation has exclusive four-coordination governed by its electronic structure.* The tetrahedral coordination concept is important, since it is necessary to distinguish the structural information revealed in the X-ray crystallography time frame and the structural information needed to trace out the mechanism of enzyme catalysis in a much shorter time frame. According to this concept, it is questionable to study zinc's coordination in proteins with Co^{2+} as a surrogate of Zn^{2+} for spectroscopic measurements, since the former is a d^7 open shell divalent cation whereas the latter is a d^{10} closed shell divalent cation. We expect that the tetrahedral coordination concept will advance understanding of the experimental data and sometimes conflicting data [24] concerning zinc proteins in crystallographic and spectroscopic studies, and offer novel insights into protein engineering and theoretical studies of zinc proteins for unlocking the secrets of Zn^{2+} in human biology.

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