A Theoretical Study of the Influence of Zn-Cd Substitution on the ^{13}C NMR Chemical Shift in Complexes that Mimetize Zinc Fingers

Teodorico C. Ramalho, Jacques F. Dias, Luiz E. Pizarro Borges and J. D. Figueroa Villar*.
Departamento de Engenharia Química, Instituto Militar de Engenharia,
Praça General Tibúrcio 80, 22290-270 Rio de Janeiro, Brazil
E-Mail: d5figuer@epq.ime.eb.br

Keyword: Zinc Finger, Theoretical calculations and ^{13}C NMR Spectroscopy.

Abstract: *MM/PM3/DFT structure calculation and ^{13}C NMR chemical shifts Ab-initio calculations at the LanL2DZ level and DFT/GIAO on the Topol Zn^{2+} and Cd^{2+} complexes explain the experimental non-variation of the chemical shifts on the basis of a mutual compensation between the electronic and structural effects of metal substitution. The results showed that extreme caution must be taken when using Cd as a probe to study the Zn coordination environment in zinc fingers and other metalloproteins.*

Resumo: *Determinação estrutural usando MM/PM3/DFT e cálculos de deslocamento químico Ab-initio a nível LanL2DZ, através do método GIAO, nos complexos de Zn^{2+} e Cd^{2+} propostos por Topol explicam a não-variação encontrada experimentalmente nos deslocamentos químicos de ^{13}C dos ligantes com base na compensação mútua entre os efeitos eletrônicos e estruturais ocorridos na substituição metálica. Os resultados sugerem que se deve ter cautela na utilização de Cd como sonda para estudos do ambiente de coordenação do Zn em dedos de zinco e outras metaloproteínas.*

Introduction

It is now well known that zinc is crucial for the synthesis of nucleic acids and, consequently, for cellular division.[1] Many important roles for this essential element have been known for a long time, but one of the more important ones was discovered only recently.[2] In 1983, the analysis of the amino acid sequence of the transcription factor TFIIIA, from the frog *Xenopus laevis*, lead Klug to the discovery of the existence of a protein containing 344 amino acid residues distributed in nine structurally very similar motifs, each containing a zinc cation.[2] Each one of those motifs had about 30 amino acid residues, among which there were always two histidines and two cysteins coordinating Zn^{2+}.[3] Further investigations demonstrated that the TFIIIA factor not only keeps Zn^{2+} while interacting with DNA, but also showed that the cation was necessary for that interaction.[4] Later, it was shown that the transcription factor TFIIIA_5S RNA also contained seven chelated zinc cations in similar motifs.[5] In 1987, Klug and co-workers showed that the zinc coordination environment in the TFIIIA motifs consisted of a tetrahedral array of four amino acid ligands, invariably two cysteines and two histidines.[6] Due to their role in binding to the major groove of DNA and to the elongated shape of the nine Zn-containing motifs from TFIIA, they called them zinc fingers.[6] Afterwards, many other similar structures were discovered, all of which functioned as transcription factors.[7-9] In all those structures, the zinc cation was always present, but sometimes the tetrahedral Zn-coordination environment was formed by different combinations of the aminoacids cysteine and histidine.[7-9]

Many metallic cations present in metaloproteins have been conveniently studied in this kind of environment by NMR techniques, especially magnesium, sodium and potassium.[10-12] Other biologically important metals are very difficult to study by NMR, especially zinc and calcium. This difficulty is due to the low natural abundance, low magnetogyric ratios and high quadrupolar properties of their NMR-detectable isotopes. Because of that, spin 7/2 ^{67}Zn, with a 4.11% natural abundance, $1.68x10^7$ rad T^{-1} s^{-1} magnetogyric ratio and quadrupole moment of 0.15x10-28 m^2, is one of the isotopes most poorly studied by NMR.[13,14] The strategy used to study the coordination environment of highly quadrupolar metals in biological systems is their substitution by electronically similar isotopes more suitable for NMR studies. In the case of ^{67}Zn, the element of choice is ^{113}Cd, as both cations have $d^{10}s^2$ electronic configurations. The first researcher

that used this approach was Armitage in 1976,[15] but since then more than 20 metaloproteins have had their zinc coordination centers studied using this method.[16-18] The most recent application of the use of the spectroscopy of ^{113}Cd RMN have been the study of the metallic centers of zinc fingers.[19-21] Despite the success of this methodology, its validity has been discussed in the literature,[22,23] the main concern being the probable distortion of the coordination environment when the harder and smaller zinc is substituted by the softer and larger cadmium. We have recently shown that Zn^{2+} tends to accommodate in a tetrahedral environment while Cd^{2+} prefers to adopt the octahedral geometry, with the participation of water molecules as ligands.[24] It was observed that when Zn^{2+} is forced into an octahedral geometry with two His-Cys and two water ligands the final geometry is halfway between octahedral and tetrahedral, with the water ligands very distant from the metal.[24] It was also observed that, as should be expected, the complexes of Cd^{2+} are more voluminous than the respective Zn^{2+} complexes.

One approach to study the effect of Zn-Cd substitution on the structure of the coordination environment is the use of molecular modeling. The objective of this work was to use DFT and *ab initio* molecular modeling methods to study the electronic and structural effects of Zn^{2+} substitution by Cd^{2+} on the ^{13}C NMR chemical shifts of a simple model of the most common two-histidine-two-cysteine coordination environment found in zinc fingers.

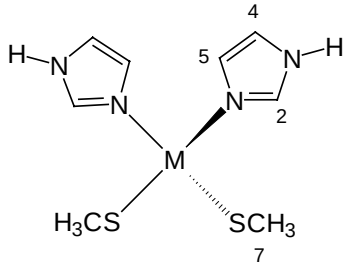
Experimental

The complexes were formed by the interaction of the Zn^{2+} and Cd^{2+} metal cations with the nitrogen atoms of two imidazoles and the sulfur atoms of two methylmercaptan ligands. Once the Zn^{2+} coordination environment is usually tetrahedral, only the complexes with a tetrahedral geometry were considered, because.[19-21] In every case, using the PC *Spartan Pro*[25] program, the first geometry optimizations were carried out by three consecutive methods: first MMFF[27] followed by PM3tm[28] and DFT. For all calculation methods, the conjugate gradient and quasi-Newton-Raphson algorithms were used for the geometry optimization until a gradient of 0.01 kcal/mol Å was obtained. The final geometries were obtained with DFT using the Becke-Perdew perturbative model with the DN* numerical polarization basis sets.[29,30] The chemical shifts were calculated using the Gaussian 98[26] program. The values were obtained with the final structures using the GIAO[31] method with the LanL2MB,[32] LanL2DZ[33] and B3LYP/6-31G**[34] basis sets (in ppm relative to the chemical shift of TMS calculated at the same level).

Results and Discussion

Coordination Environment Model

The simplest model for the zinc coordination environment in metalloproteins has been shown to be the one proposed by Ranganathan.[35] This model is composed by a Zn^{2+} cation complexed by two molecules of the bidentate dipeptide His-Cys. This complex has been shown to interact with DNA in the same way as zinc fingers.[36] Since the influence of the metal on the chemical shifts of the ligand atoms is strongly felt only two to three bonds away from the metal, we decided to use the reduced Topol model,[37] where the two cysteines are substituted by two molecules of methyl mercaptane and the two histidines are replaced by two imidazoles. The simplicity of this model also allows for the use of more complex basis sets in the calculations. According to this, the Topol complexes of Zn (**1**) and Cd (**2**) shown in Figure 1 were used as models to compare their calculated structures and the effect of the exchange of Zn by Cd on the ligands ^{13}C chemical shifts.



1: M = Zn
2: M = Cd

Figure 1. Complexes of Zn(**1**) and Cd(**2**) with two molecules of methyl mercaptane and two imidazoles.

In order to obtain the Topol models with Zn and Cd with correct geometries, we first calculated the structure of the corresponding Ranganathan models using the minimization procedure indicated in the experimental. Then, the corresponding Topol models were obtained by simply deleting the cysteine and histidine atoms in the Ranganathan complexes until two methylmercaptane and two imidazole ligands were obtained. The final structures were subjected to single point minimization. The metal-S and metal-N bond lengths obtained in this way for the Topol models were within 0.01 Å of the respective distances obtained by X-rays from the zinc finger SP2 model.[38] The results are shown in Table 1.

Table 1

Calculated bond angles and lengths in pBP86 of the Topol model for Zn and Cd and experimental X-ray data of the zinc finger SP2 model.

	Calculated	Experimental[38]
Zn-S (Å)	2.37	2.35
Cd-S (Å)	2.38	--
Zn-N (Å)	2.05	2.01
Cd-N (Å)	2.20	--
N-Zn-S (degrees)	110.5	110.3
N-Cd-S (degrees)	106.3	--

This comparison demonstrates the validity of the calculation methodology used. It is interesting to notice that the metal-S bond lengths in the tetrahedral complexes are very similar. On the other hand, the metal-N bond distances in the same complexes are very different for Zn and Cd. We believe that this behavior is due to the differences in hardness between the metals and the binding elements involved in the complex formation. It is known that Cd^{2+} is considered a soft cation, while Zn^{2+} is a borderline case with a tendency to be hard.[39] At the same time, we can consider sulfur to be soft and nitrogen to be hard. If we take into account that strong bonds are formed when there are soft-soft or hard-hard interactions, then the N-Zn bond, which possesses a hard-hard character, should be stronger and shorter than the N-Cd bond, which possesses a hard-soft nature.[39] In the case of the metal-N bonds, the fact that Cd^{2+} is larger than Zn^{2+} clearly contributes to enhance this difference in bond lengths. When we consider the metal-S bonds, since S is soft, the Zn-S bond, with hard-soft nature, should be a weaker, and therefore a longer bond, while the Cd-S bond, with soft-soft nature, should be stronger, and therefore shorter. In this case the Cd-S and Zn-S bonds are very similar in length since the stronger nature of the Cd-S bond compensates for the greater size of the Cd^{2+} cation.

Experimental Chemical Shift Values

Initially it was necessary to choose the calculation method and the basis set for the theoretical determination of the chemical shifts. In order to accomplish this, we first calculated the structure of the Zn and Cd complexes of glycine, which X-ray structures have been reported in the literature.[40] The comparison of the calculated geometries with the experimental X-ray structures indicates that the best fit was obtained with the B3LYP/6-31G** DFT method, but similar results were obtained with LanL2DZ, which required a much shorter computational time. According to this, all the calculations were carried out using *Ab-initio* with the LanL2DZ basis set. The same was observed for the chemical shifts; the best results were obtained with B3LYP/6-31G**, but similar results were obtained with LanL2DZ. The calculated values are shown in Table 2.

Table 2

Calculated ^{13}C NMR Chemical Shifts for the Topol Complexes **1** and **2**.

	1	**2**	**1-2**
Carbon	**δ ^{13}C (ppm)**	**δ ^{13}C (ppm)**	**$\Delta\delta$ (ppm)**
2	159.07	158.93	1.14
4	142.07	141.33	0.74
5	125.01	124.57	- 1.24
11	13.74	13.23	0.51

The results on Table 2 demonstrate that the differences of chemical shifts between the two complexes are very small, varying between 1.14 and -1.24 ppm. For similar complexes, the same

Table 3
Calculated ^{13}C NMR Chemical Shifts for the Topol Complexes **1**, **2** and **3**

Carbon	1 δ ^{13}C (ppm)	2 δ ^{13}C (ppm)	3 δ ^{13}C (ppm)	1-2 Δδ (ppm)	1-3 Δδ (ppm)
2	159.07	158.93	161.55	1.14	-2.48
4	142.07	141.33	145.82	0.74	-3.74
5	125.01	124.57	129.52	- 1.24	-4.51
11	13.74	13.23	14.00	0.51	-0.26

trend is found in experimental values reported in the literature.[22,41] This chemical shift non-variation is possibly due to compensation between the electronic and the structural effects due to the metal exchange.

Initially, we expected that the calculated chemical shift values for the Cd complexes to be very different from the calculated for the Zn complexes. To our surprise both the theoretical and the experimental results show very similar results for Zn and Cd complexes. This seems to corroborate the validity of Zn substitution by Cd for NMR studies. However, in our previous molecular modeling work with Ranganathan complexes of Cd and Zn, we found out that cadmium tends to form octahedral complexes as it coordinates with solvent molecules while Zn tends to remain in tetrahedral geometry.[24] Also, when the zinc is substituted by cadmium, the ligand-metal bond lengths and the volume of complex increase, indicating that there are severe modifications of the metal coordination environment.

According to this, we believe that the structural modifications are responsible for the compensation of the expected changes in chemical shifts as a consequence of the metal substitution. If this is the case, the non-variation of the chemical shifts during Zn-Cd substitution is a strong argument against the use of this kind of substitution to study by NMR the Zn coordination environment in Zn-containing proteins or any other complexes of this metal.

In order to check out the effect of the structural variation on the chemical shifts, a new Cd Topol complex (**3**) was obtained by simple substitution of the Zn in the Zn Topol complex (**1**). In this way it was possible to keep the same geometry for both complexes. When the ^{13}C chemical shifts are calculated for **3** and compared with the results for **1** (see Table 3), it can be observed that the differences increase significantly. This result is a clear indication that the experimental non-variation of the ^{13}C chemical shifts observed during Zn-Cd substitution is due to mutual compensation of the electronic and structural effects, and not to the maintenance of the coordination environment.

Conclusion

The small variation of the ^{13}C NMR chemical shifts of the ligands observed during Zn^{2+}-Cd^{2+} substitution initially seems to indicate that the substitution procedure is a valid tool to study the coordination environment of Zn. However, the results obtained in this work indicate that there are important distortions on the complex environment when Cd substitutes Zn and that the chemical shift non-variation is actually due to the mutual compensation of the structural and electronic effects of the metal substitution.

Accordingly, we recommend extreme caution when using Cd as a probe to study the Zn coordination environment in zinc fingers and other metalloproteins.

Acknowledgments

The authors wish to acknowledge the financial support given by FAPERJ, CNPq and CAPES.

References

1. Valle, B.L.; Auld, D. S., *Faraday Discuss*, **1992**, 93, 144.
2. Branden, C.; Tooze, J., Introduction to Protein Structure, Garland Publishing, New York, Chapter 5, **1991**.
3. Ginsberg, A. M.; King, B. O.; Roeder, R. G., *Cell*, **1984**, 39, 479.
4. Hanas, J. S.; Hazuda, D. J.; Wu, F. Y.; Wu, C. W., *J. Biol. Chem. Sci*., **1983,** 258, 14120.
5. Miller, J.; Mclachlan, A.; Klug, A. D., *EMBO J.*, **1985**, 4, 1609.
6. Klug, A., *Trends Biochem.Sci.*, **1987**, 12, 464.
7. Guenther, B.; Onrust, R.; Sali, A., *Cell,* **1997**,91, 335.
8. Nagadoi, A.; Nakazawa, K., *J Mol Biol.*, **1999**, 287, 593.
9. Klein, D. J.; Johnson, P. E.; Zollars, E. S., *Biochemistry*, **2000**, 39, 1604.
10. Forsen, S.; Lindman, B., *Ann. Rep. NMR Spectrosc*, **1981**, 11A, 183.
11. Laszlo, P., *Angew. Chem. Int. Edn.*, **1978**, 17, 254.
12. Cope, F. W.; Damadian, R., *Physiol. Chem. Phys.*, **1979**,11, 143.
13. Kunwar, A. C.; Turner, G. L., *J. Magn. Reson.*, **1986**, 69, 124.
14. Vosegaard, T.; Anderson, U.; H. Jakobsen, J. J., *J. Am. Chem. Soc.*, **1999**, 121, 1970.
15. Armitage, J. M.; Pajer A,. J. M., Chlebowiski, J. F.; Coleman, J. E*., J. Am. Chem. Soc.*, **1976,** 98, 5710.
16. Borden, K. L.; Lally, L. M.; Martin, S. R., *EMBO J.*, **1995**,14, 5947.
17. Kodera, Y.; Sato, K.; Tsukahara, T., *Biochemistry*, **1998**, 37, 17704.
18. Bernstein, B. E.; Hoffman, R. C.; Horvath, S., *Biochemistry*, **1994**, 33, 4460.
19. South, T. L.; Kim, B.; Sumers, M. F., J. Am. Chem. Soc., 1989, 11, 395.
20. Giedroc, D. P.; Johnson, B. A.; Armitage, I. M., Coleman, J. E., Biochemistry, 1989, 28, 2410.
21. Gardner, K. H.; Narula, T. P.; Rivera, E.; Coleman, J. E., Biochemistry, 1991, 30, 11292..
22. Low,W. B.; Hishfeld, F. L.; Richards, F. M., J. Inorg. Chem. ,1993, 81, 4412.
23. Cai, M.; Huang, Y.; Zheng, M., Protein Sci., 1998, 7, 2669.
24. Ramalho, T.C.; Figueroa-Villar, J. D., J. Molecular Structure (theochem), in press.
25. PC Spartan Pro 1.0.1, Wavefunction Inc., 18401 Von Karman Avenue, Suite 370, Irvine, CA 92612 USA.
26. Gaussian 98, Revision A.11, M. J. Frisch, et al, Gaussian, Inc., Pittsburgh PA, 1998.
27. Halgren, T. A., J. Computacional Chem., 1996, 17, 490.
28. Stewart, J.J.J., J. Computational Chem., 1989, 10, 209.
29. Becke, A. D., Phys. Rev., 1988, 38, 3089.
30. Perdew, P. J., Phys. Rev. B, 1986, 33, 8822.
31. Ditchfield, R., Mol Phys., 1974, 27, 789.
32. Hehre, W. J.; Stewart, R. F.; Pople, J. A., J. Chem. Phys., 1969, 51, 2657.
33. P. Hay, J.; Wadt; W. R., J. Chem. Phys. 1985, 82, 270 .
34. Becke, A. D., J. Chem. Phys. 1993,98, 5648 .
35. Ranganathan, S.; Jayaraman, N.; Roy, R., Tetrahedron, 1992, 48, 931.
36. Ranganathan, S.; Jayaraman, N.; Chatterji, D., Biopolymers, 1997, 41, 407.
37. Topol, I.A.; Casas-Finet, J.R. et al, J. Molecular. Strucuture (Theochem), 1998, 423, 13.
38. Narayan, V. A.; Kriwachi, W. R.; Caradonna, J. P., J. Biol. Chem., 1997, 272 7801.
39. R. G. Pearson, Hard and Soft Acids and Bases, Benchmark Papers in Inorganic Chemistry, Pennsylvania, Chapter 1,1980.
40. Merrit, L. L.; Mundy, B. W., Acta Cryst., 1954, 7, 473.
41. Chalaça, M. Z.;Figueroa-Villar, J. D.; Ellena, J. A.; Castellano, E. E,, Inorg. Chim. Acta, 2002, 328, 45-52