J. Phys. Chem. A **2008,** *112,* 12385–12392

Coordination Properties of Lysine Interacting with Co(I) and Co(II). A Theoretical and Mass Spectrometry Study

E. Constantino,[†] J. Tortajada,[‡] M. Sodupe,[†] and L. Rodríguez-Santiago*,[†]

Departament de Química, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain, and Laboratoire Analyse et Modélisation pour l'Analyse et l'Environnement, CNRS UMR 8587, Université d'Evry-Val-d'Essonne, Boulevard François Mitterrand, 91025 Evry Cedex, France

Received: July 1, 2008; Revised Manuscript Received: October 1, 2008

This article analyzes the interaction between cobalt cations and lysine both theoretically and experimentally. The influence of d orbital occupation in $Co^{+/2+}$ cations and the side chain of lysine on the relative stability of the different coordination modes was studied by means of theoretical methods. The structure and vibrational frequencies were determined using the B3LYP and BHLYP methods. Single-point calculations were also carried out at the CCSD(T) level. For both systems, Co^+−lysine and Co^{2+}−lysine, the most stable structure results from the interaction of neutral lysine to the metal cation through the two amino groups and the carbonyl oxygen, the ground electronic state being a ^3A in the case of Co^+ and ^4A for the Co^{2+} system. This is in contrast to that found for Co^{2+} interacting with glycine in which the most stable structure has the amino acid in its zwitterionic form, which points out the importance of the side chain.

Introduction

Transition-metal cations are involved in a great number of fundamental processes for living organisms. A third of all structurally characterized proteins are metalloproteins,[1] and many of them involve transition metals that are essential for their catalytic and structural properties.[2,3] However, excess concentration of different transition-metal cations such as cobalt, zinc, or nickel is toxic. Thus, the concentration of these cations has to be regulated. For example, as a response to metal toxicity, living systems have developed mechanisms of resistance based on the intracellular complexation of the toxic metal ion by peptides such as phytochelatins, which are used by plants for the storage of metal cations, mainly divalent ones such as Cu^{2+}, Zn^{2+}, or Co^{2+}.[4] A similar function is carried out in mammals by the terminal part (-Lys-Cys-Thr-Cys-Cys-Ala or Lys-Cys-Ser-Cys-Cys-Ala) of some metallothioneins, which involves the interaction of the cation with the amino acid residues. Although the major complexation sites are the Cys residues, the study of Lys complexation is also interesting since it can operate at high metal cation concentrations, and the presence of the basic side chain induces very different coordination properties compared to other more studied amino acids. On the other hand, lysine is an amino acid with a higher proton affinity.[5,6]

The noncovalent interactions between the amino acid constituents of this kind of protein and peptides with metal cations determine the structure of the molecule. A first step to understand the coordination properties of these peptides is to accurately evaluate each interaction between individual components separately. In this manner, we can gain a deep insight into the knowledge of each kind of noncovalent interaction occurring in these systems and evaluate the relative contribution of each of these interactions.

In this context, mass spectrometry and theoretical methods are very valuable techniques for the study of metal cation-biomolecule intrinsic properties. In particular, extensive work has been done to investigate the influence of transition-metal cations on the stability of the different forms of amino acids and small peptides, which is reflected in the large number of publications, experimental and theoretical, dedicated to this topic.[7−64] However, while the interaction of amino acids such as glycine,[7,9,11,13−16,18−21,25,27−31,34,36,48,60,63] alanine,[10,11,13,15,18−22,34,35,37,40] cysteine,[11,13,15,18−20,34,37,39] or aromatic amino acids[8,11,13,15,18−20,26,34,35,64] with transition-metal cations has been extensively studied, the number of works devoted to the interaction with lysine is smaller, most of them focusing on metal cation-amino acid affinities.[11−13,15,18−20,34,35,37,62]

To the best of our knowledge, only the interaction of lysine with Ag^+ and alkali metal cations has been considered.[34,62] The lowest energy form of Ag^+−lysine has predicted to be non-zwitterionic. However, for the alkali metal cations the most stable conformer varies depending on the size of the cation. Other works on the interaction of metal cations with lysine-containing peptides have explored the fragmentation patterns of these complexes in mass spectrometry experiments.[38,42]

In the present study, we show the mass spectrum obtained from an electrosprayed solution of Co^{2+} with lysine jointly with a detailed theoretical analysis of the gas-phase binding chemistry between Co^+ and Co^{2+} cations and lysine, which is one of the most basic amino acids. The ground electronic states of Co^+ and Co^{2+} are $^3F(3d^8)$ and $^4F(3d^7)$, respectively. Because of their open shell nature, the interaction of these cations with amino acids can lead to several low-lying electronic states arising from different metal d occupation. Moreover, depending on the degree of metal complexation, the relative stability of different spin electronic states could vary. Thus, in addition to the triplet states derived from the interaction of the $^3F(3d^8)$ ground state of Co^+, we also considered the singlet state that arises from the ^1G $(3d^8)$ excited state of Co^+. The quintet state, arising from the (s^1d^7) ^5F state of Co^+, has not been considered since, as stated in our previous work on Co^+−glycine,[63] and shows a much larger repulsion between the metal and the ligand because of the 4s occupation of the metal. Thus, it is reasonable to expect that

---

* Corresponding author. E-mail: luis@klingon.uab.es.

[†] Universitat Autonoma de Barcelona.

[‡] Université d'Evry-Val-d'Essonne.

10.1021/jp805764y CCC: \$40.75

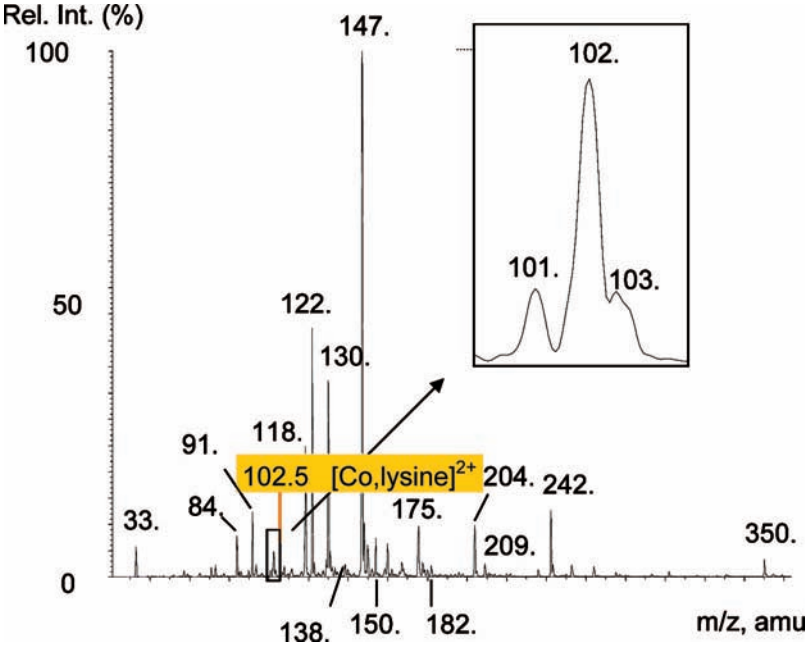

Figure 1. ESI source spectrum of the solution of lysine and $CoSO_4$ in water/methanol 1:1 solvent.

the increase in the number of basic centers coordinated to the metal cation will result in a larger destabilization of the quintet state.

Experimental Section

Electrospray mass spectrum was recorded on an Applied Biosystems/MDS Sciex API2000 triple-quadrupole instrument fitted with a "turboionspray" ion source. Sample was introduced in the source using direct infusion with a syringe pump, at a flow rate of 10 μL/min. Ionization of the samples was achieved by applying a voltage of 5.0 kV on the sprayer probe and by using a nebulizing gas (GAS1, air) surrounding the sprayer probe, intersected by a heated gas (GAS2, air) at an angle of approximately 90°. The operating pressure of GAS1 and GAS2 was adjusted to 2.0 bar, by means of an electronic board (pressure sensors), as a fraction of the air inlet pressure. The curtain gas (N_2), which prevents air or solvent from entering the analyzer region, was similarly adjusted to a value of 2.0 bar. The temperature of GAS2 was set to 70 °C. The declustering potential (DP), defined as the difference of potentials between the orifice plate and the skimmer (grounded), typically referred to as the "cone voltage" for other electrospray interfaces, was adjusted to 5 V to give the maximum intensity of the peak of interest.

Lysine and cobalt salts were purchased from Aldrich and were used without further purification. Sample solutions were prepared from $CoSO_4 \cdot H_2O$/amino acid mixture with concentrations of 10^{-4} mol L^{-1} in a methanol/water 1:1 solvent.

Methods

Molecular geometries and harmonic vibrational frequencies of the considered structures were obtained using the nonlocal hybrid three-parameter B3LYP density functional approach,[65−67] as implemented in the Gaussian 03 set of programs package. Previous theoretical calculations showed that theB3LYP approach is a cost-effective method for studying transition-metal ligand systems.[68−71] However, recent studies carried out in our group demonstrated that, for systems where the spin delocalization is important, functionals with a larger percentage of exact exchange, such as BHLYP,[72] provide better results compared to the highly correlated CCSD(T) method. Thus, for the most stable structures, in addition to B3LYP, we also carried out calculations with BHLYP. Moreover, to confirm the reliability of the DFT results for the $Co^+{-}L$ and $Co^{2+}{-}L$ systems, we performed calibration calculations for selected structures using the single and double coupled cluster method with a perturbational estimate of the triple excitations CCSD(T).[73] In these calculations, all valence electrons were correlated.

Geometry optimizations and frequency calculations were performed using the following basis set: the Co basis is based on the (14s9p5d) primitive set of Wachters[74] supplemented with one s, two p, one d diffuse functions,[75] and two f polarization functions,[76] the final contracted basis set being [10s7p4d2f]. For C, N, O, and H we used the 6-31++G(d,p) basis set. Thermodynamic corrections were obtained assuming an ideal gas, unscaled harmonic vibrational frequencies, and the rigid rotor approximation by standard statistical methods.[77] Net atomic charges and spin densities were obtained using the natural population analysis of Weinhold et al.[78,79] Open shell calculations were performed using an unrestricted formalism. All calculations were performed with the Gaussian 03 package.[80]

Results and Discussion

The electrospray spectrum of the cobalt sulfate/lysine mixtures is dependent on the cone voltage, also referred to as DP in our instrument. The larger intensity of the peak corresponding to Co^{2+}−lysine ($m/z = 102.5$) was obtained at DP = 5 V. Figure 1 shows the source spectrum recorded at this value of the declustering potential. The observation of this complex is in contrast to what was found for glycine, since the complex Co^{2+}−glycine was not observed.[81] This fact shows the important role of the lateral chain of lysine in the complexation of the metal cation. The reduced complex Co^+−lysine was not observed under electrospray conditions, showing that no reduction of the metal cation takes place in this case, in contrast to what was observed for other metals such as Cu^{2+} under the same conditions.[26] This is because the most stable oxidation states of Co in solution are +2 and +3. In electrospray, since the

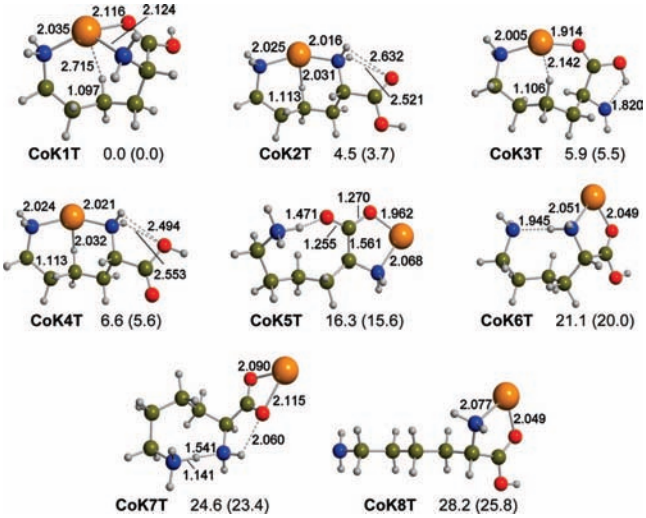

Figure 2. B3LYP-optimized geometries for the different minima of Co^+−lysine in the triplet state and relative energies including zero point corrections (ΔG^0_{298} in parentheses). Distances are in angstroms, and energies are in kilocalories per mole.

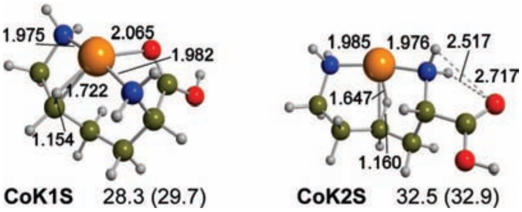

Figure 3. B3LYP-optimized geometries for the low-lying conformers of Co^+−lysine in singlet state and relative energies including zero point corrections calculated with respect to the triplet global minimum (ΔG^0_{298} in parentheses). Distances are in angstroms, and energies in are kilocalories per mole.

complexes are obtained from a solution of a Co(II) salt, the +1 state is not produced. However, other techniques such as fast atom bombardment allow obtaining Co(I) complexes such as Co^+−adenine.[82] Therefore, and because in biological systems transition-metal ions can be found in multiple oxidation states, including the +1 charge state for cobalt,[83] we included the theoretical study of the singly charged complex.

Co^+−Lysine System. As commented above, we considered the states arising from the (d^8) ^3F and (d^8) ^1G states of Co^+. These states can interact with the ^1A state of lysine leading to a ^3A and a ^1A electronic state of the Co^+−lysine complex. In addition to the basic centers found in glycine, lysine has a fourth basic center and a very flexible side chain that leads to a much more complicated conformational pattern. To explore the conformational space of this kind of system, a previous search of the Li^+−lysine system was performed to model the electrostatic interaction of the metal cation with the amino acid. This primary study was carried out using the Monte Carlo multiple minimum procedure,[84] with the AMBER* force field[85,86] as implemented in the Macromodel 7.0 package.[87] Moreover, some structures not obtained in this initial conformational search but chemically important and derived from experience and chemical intuition were also computed. Figures 2 and 3 show the obtained minima for the different conformers considered in the triplet and singlet states, respectively. Singlet and triplet conformers are similar and for the sake of brevity only the two low-lying structures of the singlet state are shown. The other considered structures can be found in Figure S1 of the Supporting Information.

Computed relative energies as well as natural population analysis of the metal cation are shown in Table 1.

It has been shown[63] that the singlet−triplet separation for Co^+-containing systems is somewhat overestimated at the B3LYP level. This overestimation is already observed for free Co^+ and deviation carries over the molecular system. Therefore, we corrected the relative energies considering the experimental values for free Co^+ (see ref 63). The value of this correction is −8.5 kcal mol^{-1}, which is the difference between the experimental singlet−triplet separation and that calculated at the B3LYP level.

It should be noted that the considered structures correspond to the most stable ones for each coordination environment, that is, $N_lN_tO_c$, N_lN_t, N_lO_c, N_tO_c, N_tO^-, OO^-, where N_l is the amino nitrogen of the side chain, N_t is the terminal amino nitrogen, O_c is the carbonyl oxygen, and O^- is the carboxylate oxygen of the zwitterionic form of the amino acid.

All the structures show C_1 symmetry and can be classified into two main groups depending on whether the side chain interacts with the metal cation. That is, group I includes structures **CoK1−CoK4** where the amino group of the side chain takes part in the coordination environment of the metal cation. Group II comprises structures **CoK5−CoK8** where only the backbone basic groups of the amino acid coordinate to the Co cation. In the first group structures, metal coordination involves eight- or nine-member rings, whereas in the second case the coordination environment of the metal cation is very similar to that found for glycine, characterized by the formation of four- and five-member rings. This latter kind of coordination is frequently found in metallated peptides.[88]

Table 1 shows that the triplet states of Co^+−lysine are more stable than the singlet ones as it happens for free Co^+ and Co^+−glycine. However, the singlet−triplet difference for a given conformation is somewhat smaller in Co^+−lysine (between 21.4 and 36.0 kcal mol^{-1}) than in Co^+−glycine (between 25.8 and 38.5 kcal mol^{-1}). For both spin states, the most stable structure (**CoK1T** and **CoK1S**) corresponds to the metal cation interacting with the two amino nitrogen atoms and the carbonyl oxygen of neutral lysine. This structure was also proposed for the interaction of lysine with Ag^{+34} and small alkali metal cations such as Li^+ and Na^+. However, for K^+, with larger ionic radii, the preferred structure corresponds to the coordination of a zwitterionic structure.[62] In addition, for both spin states the structures where the side chain amino group is coordinated to the metal cation (group I) are more stable than the structures where this center is not coordinated (group II) because of the larger basicity of the side chain amino group. On the other hand, if we take as the *yz* plane the one defined by the metal cation and the two atoms directly bonded to it (or in the case of **CoK1T** and **CoK1S** the plane defined by Co, the carbonyl oxygen, and the side chain nitrogen atom), the highest singly occupied orbital in the triplet states corresponds to the antibonding combination of the d_{z^2} orbital (group I structures) or the d_{yz} orbital (group II) with the lone pairs of the atoms of the ligand. This was to be expected considering that occupation of that orbital would lead to a high Pauli repulsion energy. Moreover, to reduce repulsion, this orbital polarizes through sd hybridization (group I) or pd hybridization (group II). As shown in Table 1, the sd hybridization is much more effective than the pd one, since there is an important occupation of the 4s orbital for the structures of group I (around 0.4 electrons), while the occupation of the 4p orbital of the metal is always around 0.01 for all the structures. In the singlet states, this repulsive orbital is empty which allows a more important donation from the ligand to the metal cation as

TABLE 1: Relative Energies Including Zero Point Corrections of Co^+−Lysine (Kilocalories per Mole) and Population Analysis at the B3LYP Level

				Co natural population			
structure	coordination	state	ΔE	4s	3d	q(Co)	spin(Co)
CoK1T	N_l, N_t, O_C	^3A	0.0	0.34	7.89	0.76	1.93
CoK2T	N_l, N_t	^3A	4.5	0.42	7.85	0.71	1.96
CoK3T	N_l, O_C	^3A	5.9	0.43	7.80	0.76	1.96
CoK4T	N_l, N_t	^3A	6.6	0.42	7.85	0.71	1.96
CoK5T	N_t, O_C	^3A	16.3	0.22	7.94	0.82	1.91
CoK6T	N_t, O_C	^3A	21.1	0.13	8.00	0.85	1.94
CoK7T	O, O^-	^3A	24.6	0.18	7.97	0.84	1.95
CoK8T	N_t, O_C	^3A	28.2	0.12	8.00	0.87	1.95
CoK1S	N_l, N_t, O_C	^1A	28.3	0.24	8.17	0.57	0.00
CoK2S	N_l, N_t	^1A	32.5	0.24	8.16	0.59	0.00
CoK4S	N_l, N_t	^1A	34.6	0.24	8.16	0.59	0.00
CoK5S	N_t, O_C	^1A	37.6	0.36	8.01	0.61	0.00
CoK3S	N_t, O_C	^1A	41.9	0.37	7.94	0.68	0.00
CoK6S	N_t, O_C	^1A	45.0	0.32	8.02	0.65	0.00
CoK7S	O, O^-	^1A	52.6	0.34	8.00	0.65	0.00
CoK8S	N_t, O_C	^1A	53.2	0.30	8.02	0.67	0.00

reflected by the values of the metal cation charges shown in Table 1. This donation is somewhat larger in the structures of group I than in group II.

In all the conformers except **CoK1T** and **CoK1S**, the metal cation is interacting with only two basic centers of the amino acid. The energy ordering and the energy differences between the isomers are very similar for both spin states. The only exception corresponds to **CoK3**, which is 5.9 kcal mol^{-1} less stable than the ground triplet state **CoK1T** but 13.6 kcal mol^{-1} higher in energy than the most stable isomer of the singlet state, **CoK1S**.

Structures **CoK5** and **CoK7** of both spin multiplicities deserve a separate comment since they are the only ones where the metal cation interacts with the zwitterionic form of the amino acid. In both cases, the protonation site is the amino group of the side chain and they differ in the metal cation interaction. In **CoK5**, the interaction takes place through the carboxylic oxygen and the amino nitrogen of the backbone, resulting in a five-member ring. In the second case (**CoK7**), the metal cation interacts with the carboxylate group forming a more strained four-member ring and consequently less stabilized.

As mentioned, the highest d metal orbital in the system is empty in the singlet states, resulting in shorter Co−lysine distances compared to those in the triplets. The changes between singlet and triplet differences are more important for the metal ligand distances of group II structures ($\Delta d_{T-S} = 0.125-0.184$ Å) than for the group I ones ($\Delta d_{T-S} = 0.039-0.092$ Å). This is because in the group I triplet structures the sd hybridization reduces the electron density in the internuclear axis allowing the shortening of the bonds.

Another aspect different from that found for the Co^+−glycine system is the presence of agostic interactions in the coordination of lysine to Co^+, that is, the interaction between the cobalt cation and a σ C−H bond. It can be observed in Figures 2 and 3 that this kind of interaction, found in structures of group I, is established always with the γ carbon and is more important for the singlet than for the triplet states. The Co−H distances range from 1.647 to 1.722 Å in the singlets and from 2.031 to 2.142 Å in the triplets. As a consequence, the C−H distances in the singlets (1.154−1.160 Å) become larger than in the triplet states (1.106−1.113 Å). This kind of interaction was observed before for Cu^+ and Ni^+ complexes[89−92] and was interpreted in terms of donation from the C−H σ bonding orbital to the vacant orbitals of the metal cation and a back-donation from a filled orbital of the metal to the C−H σ^* antibonding orbital. This interpretation also applies in our case. For example, the second-order NBO orbital interaction analysis for structure **CoK2S** shows a donation from the C_γ−H σ bonding orbital to the vacant 3d and 4s orbitals of Co associated with an interaction energy of 33 kcal mol^{-1}. Simultaneously, there is a back-donation from a filled d orbital of Co toward the C_γ−H σ^* antibonding orbital associated with an interaction energy of 10 kcal mol^{-1}.

HCo^+(Lys−H) Transferred Structures. The presence of agostic interactions made us think about the possibility of finding structures arising from the migration of one H atom from the interacting σ bond to the metal cation. We considered all the structures derived from the transfer of the γ or δ hydrogen atom to the metal cation. Figure 4 shows the optimized geometries and Table 2 the computed relative energies as well as the charge and spin of the metal cation derived from natural population analysis. For the sake of brevity, we show only the most stable structure of the two possible ones for each type of coordination. It can be observed that, in all cases, in addition to the bonds between the metal atom and the basic centers, two new bonds are established between the metal ion and the H and C atoms involved in the previous agostic interaction. Moreover, all the structures show a similar coordination environment around the metal, that is, octahedral-like with one or two vacant positions. The obtained structures are always less stable that the ground-state structure for Co^+−lysine (**CoK1T**). However, while the transferred triplet state structures are higher in energy than the corresponding nontransferred structures (between 27.8 and 36.0 kcal mol^{-1}), the transferred singlet structures are more stable than the nontransferred ones (between 9.1 and 16.3 kcal mol^{-1}). As a result, there is an inversion of the relative stability of both states in the transferred structures, the singlets becoming more stable than the triplets. This was to be expected if one considers that the transfer of the hydrogen atom increases in two the number of ligands attached to the metal center, which results in a larger destabilization of the triplet state in front of the singlet state, with an empty d orbital.

Co^{2+}−Lysine System. As in the case of Co^{2+}−glycine, we considered only the quartet spin state (d^7) of Co^{2+} since the doublet states lie much higher in energy and coordination is not expected to reverse the doublet-quartet relative energy. To find the most stable structures for each coordination environment, we followed the same procedure as for Co^+−lysine. Figure 5 and Table 3 show the optimized geometries for the

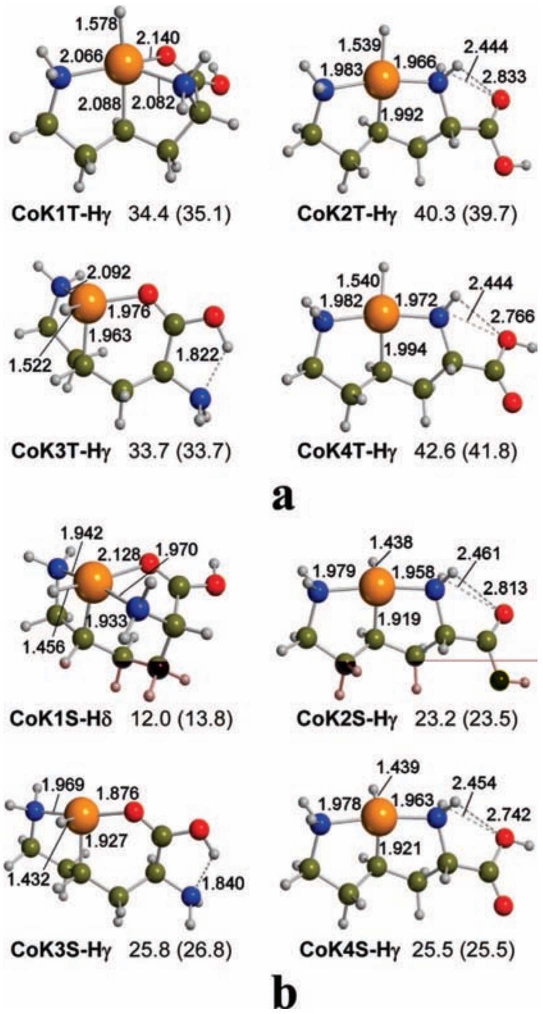

Figure 4. B3LYP-optimized geometries for the different minima of the triplet (a) and singlet (b) states of the transferred structures of Co^+−lysine and relative energies including zero point corrections (ΔG^0_{298} in parentheses). Distances are in angstroms, and energies are in kilocalories per mole.

different localized minima and the computed relative energies and the natural population analysis of the metal cation. The obtained isomers are very similar to those found for Co^+−lysine. The only difference is the absence of a **CoK6** type structure and the presence of **CoK9Q**, which was obtained when optimizing the **CoK6**-type structure in the quadruplet state. During the optimization process, the H atom of the amino terminal group was spontaneously transferred to the side chain amino group the structure evolving to **CoK9Q** to reduce repulsion between the positively charged NH_3^+ and the metal cation. Such proton transfer responds to an increase of acidity of the NH_2 group upon coordination to Co^{2+}.

The most stable structure of Co^{2+}−lysine, **CoK1Q**, shows the same type of coordination as the monocharged complex. This is different from that found for other amino acids such as glycine, where the interaction with the divalent cation preferentially stabilizes a zwitterionic structure. In the case of lysine, the interaction with the third basic center and the agostic interaction compensate the enhanced electrostatic interaction between the carboxylate group and the doubly charged metal cation in the zwitterionic structure. This fact illustrates the great importance of the side chain in the stabilization of the neutral forms of the amino acid upon the interaction with metal cations. The following structures in order of energy correspond to two zwitterionic ones, **CoK5Q** and **CoK9Q**, thus showing a very different order to that found for the monocharged systems. On the other hand, the metal charge of the Co atom (Table 3) indicates that charge transfer from the ligand to the metal cation is somewhat more important than in the case of the singly charged complexes. However, the spin remains mostly localized over the metal cation. This charge transfer is especially important in the case of **CoK8Q** for which an oxidation of the side chain at the amino group takes place. Because of this oxidation, the amino group becomes almost planar and the conformation of lysine in this structure minimizes the repulsion between the oxidized amino group and the metal cation.

As found for the $Co^{+/2+}$−glycine complexes, the metal ligand distances in Co^{2+}−lysine are shorter than the distances in the triplet state of Co^+−lysine because of the enhancement of the electrostatic interaction. However, the Co^{2+}−lysine distances are larger than the distances in the singlet state structures because of the occupation of the most repulsive orbital in the Co^{2+}−lysine system, which leads to a larger Pauli repulsion. Once more, we find in Co^{2+}−lysine the agostic interactions described for the monocations in all the structures of group I (**CoK1Q−CoK4Q**). Optimized geometries indicate that these interactions are larger for Co^{2+} than for Co^+. However, the transferred structures in the case of the dication lie more than 50 kcal mol^{-1} higher in energy than **CoK1Q** and consequently will not be presented.

Finally, Figure 6 shows the IR spectrum of the most stable structure of Co^{2+}−lysine. The vibrational frequencies have been scaled by 0.96.[93] Several features can be emphasized: the coordination to the carbonylic oxygen induces a red shift of 179 cm^{-1} with respect to that in free lysine (Figure S2 of the Supporting Information), leading to an intense C═O stretching band at 1585 cm^{-1}; the band corresponding to the COH out-of-plane bending, found at 709 cm^{-1}, is also red-shifted by 153 cm^{-1} because of the loss of the OH···NH_2 hydrogen bond in the complex; the broadband at 3288 cm^{-1} comprises the symmetric and asymmetric NH_2 stretchings of both amino groups which are shifted to lower energies by about 80 cm^{-1} because of their coordination to the metal cation. All these shifts are significantly larger than those found for singly charged

TABLE 2: Relative Energies Including Zero Point Corrections of the Transferred Structures of Co^+−Lysine (Kilocalories per Mole) and Population Analysis at the B3LYP Level, with Respect to the Most Stable, CoK1T, Singly Charged Structure

				Co natural population			
structure	coordination	state	Δ*E*	4s	3d	*q*(Co)	spin(Co)
CoK3T−Hγ	N_l, O_C, C, H	^3A	33.7	0.41	7.51	1.06	2.10
CoK1T−Hγ	N_l, N_t, O_C, C, H	^3A	34.4	0.37	7.39	1.21	2.10
CoK2T−Hγ	N_l, N_t, C, H	^3A	40.3	0.42	7.56	0.99	1.67
CoK4T−Hγ	N_l, N_t, C, H	^3A	42.6	0.42	7.57	0.99	1.67
CoK1S−Hδ	N_l, N_t, O_C, C, H	^1A	12.0	0.35	7.98	0.65	
CoK2S−Hγ	N_l, N_t, C, H	^1A	23.2	0.38	7.99	0.62	
CoK4S−Hγ	N_l, N_t, C, H	^1A	25.5	0.38	7.99	0.62	
CoK3S−Hγ	N_l, O_C, C, H	^1A	25.8	0.36	7.94	0.69	

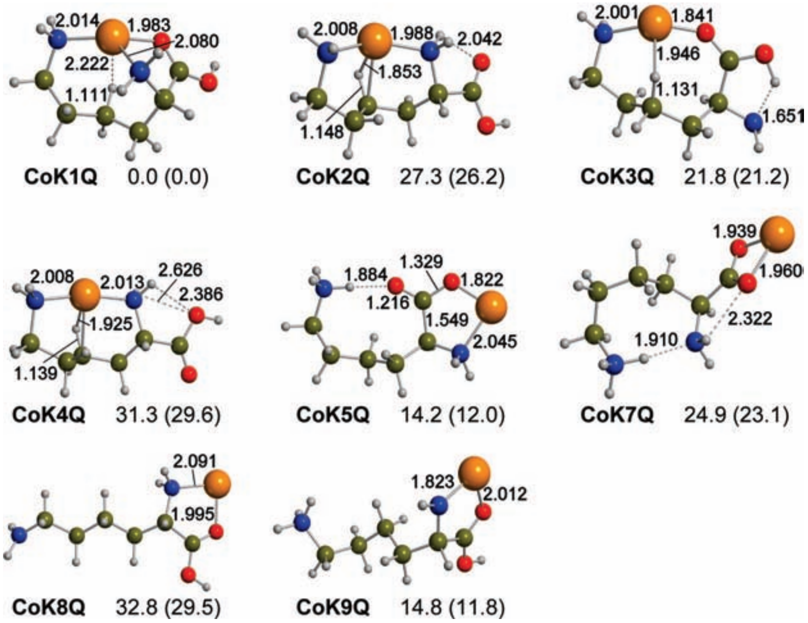

Figure 5. B3LYP-optimized geometries for the different minima of Co^{2+}−lysine and relative energies including zero point corrections (ΔG^0_{298} in parentheses). Distances are in angstroms, and energies are in kilocalories per mole.

TABLE 3: Relative Energies Including Zero Point Corrections of Co^{2+}−Lysine (Kilocalories per Mole) and Population Analysis at the B3LYP Level

				Co natural population			
structure	coordination	state	ΔE	4s	3d	q(Co)	spin(Co)
CoK1Q	N$_t$, N$_l$, O$_C$	^{4}A	0.0	0.26	7.25	1.46	2.74
CoK5Q	N$_t$, O$_C$	^{4}A	14.2	0.18	7.33	1.48	2.64
CoK9Q	N$_t$, O$_C$	^{4}A	14.8	0.19	7.49	1.30	2.46
CoK3Q	N$_l$, O$_C$	^{4}A	21.8	0.24	7.26	1.49	2.73
CoK7Q	O, O$^-$	^{4}A	24.9	0.17	7.31	1.50	2.68
CoK2Q	N$_t$, N$_l$	^{4}A	27.3	0.30	7.24	1.44	2.76
CoK4Q	N$_t$, N$_l$	^{4}A	31.3	0.30	7.22	1.45	2.81
CoK8Q	N$_t$, O$_C$	^{4}A	32.8	0.12	7.75	1.12	2.22

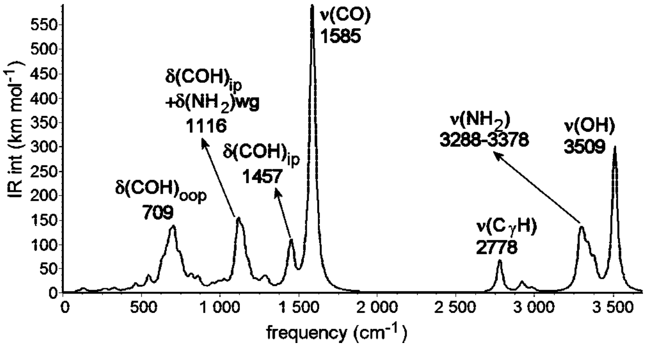

Figure 6. Computed infrared spectrum for the most stable structure of Co^{2+}−lysine (**CoK1Q**).

TABLE 4: Interaction Energies (D_e, D_0, ΔH^0_{298}, ΔG^0_{298}) (Kilocalories per Mole) of Co$^+$−Lysine and Co^{2+}−Lysine

		Co(I)	Ag(I)[a]	Co(II)
D_e	B3LYP	107.8		278.8
	BH and HLYP	99.2		265.0
	CCSD(T)	109.9[b]		271.8
D_0		106.3[c]		267.6[c]
ΔH^0_{298}		107.8[d]	71.0	269.6[d]
ΔG^0_{298}		95.3[d]	62.4	256.0[d]

[a] Reference 34. [b] Computed at the B3LYP geometry. [c] Determined using the CCSD(T) value and the B3LYP unscaled harmonic frequencies. [d] After taking into account thermal corrections at the B3LYP level.

transition metals interacting with amino acids[64] pointing out the importance of the electrostatic effects. The band found at 2778 cm^{-1} corresponding to the C$_\gamma$H stretching is also remarkable, and it is red-shifted by 104 cm^{-1}, confirming the agostic interaction between the C$_\gamma$−H bond and the metal.

Interaction Energies. Table 4 shows the computed interaction energies for the most stable structure of Co$^+$−lysine and Co^{2+}−lysine. In both cases, the coordination of the basic side chain of lysine to the metal cation results in an increase in the interaction energy. Thus, for the monocation, at the B3LYP level, the lysine metal cation affinity (ΔH^0_{298}(Co$^+$−Lys) = 107.8 kcal mol^{-1}) is 37.5 kcal mol^{-1} larger than that of glycine (ΔH^0_{298}(Co$^+$−Gly) = 70.3 kcal mol^{-1}), whereas for the dication the affinity increases 87.4 kcal mol^{-1} from glycine (ΔH^0_{298}(Co^{2+}−Gly) = 189.2 kcal mol^{-1}) to lysine (ΔH^0_{298}(Co^{2+}−Lys) = 276.6 kcal mol^{-1}). On the other hand, the metal cation affinity for Co$^+$ is larger than the previously reported value for Ag$^+$ because of the large radius of Ag$^+$ and the complete occupation of the d orbitals in this metal cation, which leads to longer metal−ligand distancies, thereby reducing the electrostatic interaction.

It can be observed that the values at the B3LYP level are somewhat larger than those at the BHLYP one. Recent studies in our group[94,95] showed that systems with an important spin delocalization functionals with a larger amount of exact exchange, such as BHLYP, compare better with the CCSD(T) results than GGA or functionals with a small percentage of exact exchange such as B3LYP. The reason is that these latter functionals overestimate delocalized situations, as a result of a bad cancelation of the self-interaction part by the exchange-correlation functional.[96] However, in the case of Co^+—lysine, the spin delocalization is small (Table 1) and the value computed at the B3LYP level is in excellent agreement with the CCSD(T) one. In the case of Co^{2+}, the difference between both functionals is somewhat larger than for Co^+ but, since spin density in **CoK1Q** is mainly located at the metal atom, the B3LYP result is reasonably accurate compared to the CCSD(T) one. In fact, the CCSD(T) value lies just in between the B3LYP and BHLYP values.

Conclusions

The reaction of cobalt sulfate with lysine in the electrospray source leads to the formation of the Co^{2+}—lysine complex. However, the Co^+—lysine one is not observed, indicating that no reduction of the metal cation is produced at the experimental conditions.

In the computational study of the binding of cobalt cations, Co^+ and Co^{2+}, to lysine, several coordination modes as well as different electronic states arising from the triplet and singlet ($3d^8$) states of Co^+ and quartet states ($3d^7$) of Co^{2+} were considered.

For Co^+—lysine, the ground-state structure is found to be tridentate through both amino groups and the carbonyl oxygen of neutral lysine, the ground electronic state being ^3A. Although lysine has one more basic center than glycine, which leads to a higher coordination of the metal atom, the singlet—triplet difference is not significantly reduced from that found for Co^+—glycine. For both spin states, the structures with the amino group of the side chain interacting with the metal cation are more stable than those with the terminal amino group. This is due to the larger basicity of the side chain amino group and the possibility of reducing repulsion through sd hybridization in the former structures. The relative energy of the different conformers follows the same order in both spin states: the zwitterionic triplet-state structures lie 16—25 kcal mol^{-1} higher in energy than the ground-state structure and the zwitterionic singlet ones are 9—24 kcal mol^{-1} higher than the most stable singlet conformer.

In all the considered structures of the Co^+—lysine system, the d orbital having a larger overlap with lysine is empty for singlets and singly occupied for triplets, leading to smaller metal—ligand distances in the case of singlet states due to smaller metal—ligand repulsion. The presence of agostic interactions between the metal cation and a σ C—H bond of the ligand in the structures where the side amino group is coordinated to the metal center is remarkable. However, transferred structures resulting from the migration of the H atom involved in the agostic interaction to the metal are always higher in energy than the most stable nontransferred one (**CoK1T**), although an inversion of the relative stability of the spin states takes place. That is, for the transferred structures the singlet structures become more stable than the triplet ones.

For Co^{2+}—lysine, the most stable structure shows the same coordination environment around the metal than for Co^+, that is, Co^{2+} interacts with neutral lysine through the two amino groups and the carbonyl oxygen. This is in contrast to what was found for glycine, for which the most stable structure was found to arise from the interaction of the zwitterionic form of the amino acid. This fact is due to the Co^{2+} interaction with the side chain of lysine and the presence of agostic interactions in the neutral form. For both metal cations, the computed binding energies show larger values than those previously reported for glycine because of the coordination of the third basic center corresponding to the side chain.

Acknowledgment. Financial support from MCYT and DURSI, through the CTQ2005-08797-C02-02/BQU and SGR2005-00244 projects, and the use of the Catalonia Supercomputer Centre (CESCA) are gratefully acknowledged.

Supporting Information Available: Optimized geometries for all minima of Co^+—lysine in singlet state and computed infrared spectrum of lysine. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Finney, L. A.; O'Halloran, T. V. *Science* **2003**, *300*, 931.

(2) Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, 1994.

(3) *Probing of Proteins by Metal Ions and Their Low-Molecular-Weight Complexes*; Sigel, A., Sigel, H., Eds.; Metal Ions in Biological Systems 38; Marcel Dekker: New York, 2001.

(4) Cobbett, C. S. *Plant Physiol.* **2000**, *123*, 825.

(5) Bliznyuk, A. A.; Schaefer, H. F., III; Amster, I. J. *J. Am. Chem. Soc.* **1993**, *115*, 5149.

(6) Maksic, Z. B.; Kovacevic, B. *Chem. Phys. Lett.* **1999**, *307*, 497.

(7) Ai, H.; Bu, Y.; Han, K. *J. Chem. Phys.* **2003**, *118*, 10973.

(8) Barlow, C. K.; Moran, D.; Radom, L.; McFadyen, W. D.; O'Hair, R. A. J. *J. Phys. Chem. A* **2006**, *110*, 8304.

(9) Bertran, J.; Rodríguez-Santiago, L.; Sodupe, M. *J. Phys. Chem. B* **1999**, *103*, 2310.

(10) Bouchonnet, S.; Hoppilliard, Y.; Ohanessian, G. *J. Mass Spectrom.* **1995**, *30*, 172.

(11) Cerda, B. A.; Wesdemiotis, C. *J. Am. Chem. Soc.* **1995**, *117*, 9734.

(12) Cerda, B. A.; Wesdemiotis, C. *Int. J. Mass Spectrom.* **1999**, *185—187*, 107.

(13) Gatlin, C. L.; Turecek, K.; Vaisar, T. *J. Am. Chem. Soc.* **1995**, *117*, 3637.

(14) Hoppilliard, Y.; Rogalewicz, F.; Ohanessian, G. *Int. J. Mass Spectrom.* **2000**, *204*, 267.

(15) Hoyau, S.; Ohanessian, G. *J. Am. Chem. Soc.* **1997**, *119*, 2016.

(16) Hoyau, S.; Pélicier, J.-P.; Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. *Eur. J. Mass Spectrom* **2001**, *7*, 303.

(17) Lavanant, H.; Hecquet, E.; Hoppilliard, Y. *Int. J. Mass Spectrom.* **1999**, *185—187*, 11.

(18) Lavanant, H.; Hoppilliard, Y. *J. Mass Spectrom.* **1997**, *32*, 1037.

(19) Lee, V. W.-M.; Li, H.; Lau, T.-C.; Guevremont, R.; Siu, K. W. M. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 760.

(20) Lei, Q. P.; Amster, I. J. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 722.

(21) Marino, T.; Russo, N.; Toscano, M. *J. Inorg. Biochem.* **2000**, *79*, 179.

(22) Marino, T.; Russo, N.; Toscano, M. *J. Mass Spectrom.* **2002**, *37*, 786.

(23) Marino, T.; Toscano, M.; Russo, N.; Grand, A. *J. Phys. Chem. B* **2006**, *110*, 24666.

(24) Pulkkinen, S.; Noguera, M.; Rodríguez-Santiago, L.; Sodupe, M.; Bertran, J. *Chem.—Eur. J.* **2000**, *6*, 4393.

(25) Remko, M.; Rode, B. M. *J. Phys. Chem. A* **2006**, *110*, 1960.

(26) Rimola, A.; Sodupe, M.; Tortajada, J.; Rodríguez-Santiago, L. *Int. J. Mass Spectrom.* **2006**, *257*, 60.

(27) Rodriguez-Santiago, L.; Sodupe, M.; Tortajada, J. *J. Phys. Chem. A* **2001**, *105*, 5340.

(28) Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. *Int. J. Mass Spectrom.* **2000**, *201*, 307.

(29) Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. *Int. J. Mass Spectrom.* **2001**, *206*, 45.

(30) Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. *Int. J. Mass Spectrom.* **2003**, *227*, 439.

(31) Rogalewicz, F.; Ohanessian, G.; Gresh, N. *J. Comput. Chem.* **2000**, *21*, 963.

(32) Rulísek, L.; Havlas, Z. *J. Am. Chem. Soc.* **2000**, *122*, 10428.

(33) Rulísek, L.; Havlas, Z. *J. Phys. Chem. B* **2003**, *107*, 2376.

(34) Shoeib, T.; Siu, K. W. M.; Hopkinson, A. C. *J. Phys. Chem. A* **2002**, *106*, 6121.

(35) Wen, D.; Yalcin, T.; Harrison, A. G. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1155.

(36) Xu, Y.; Zhang, X.; Yergey, A. L. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 25.

(37) Yalcin, T.; Wang, J.; Wen, D.; Harrison, A. G. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 749.

(38) Yu, S.; Lee, S.; Chung, G.; Oh, H. *Bull. Korean Chem. Soc.* **2004**, *25*, 1477.

(39) Spezia, R.; Tournois, G.; Cartailler, T.; Tortajada, J.; Jeanvoine, Y. *J. Phys. Chem. A* **2006**, *110*, 9727.

(40) Chu, I. K.; Guo, X.; Lau, T.-C.; Siu, K. W. M. *Anal. Chem.* **1999**, *71*, 2364.

(41) Hu, P.; Loo, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 11314.

(42) Kish, M. M.; Wesdemiotis, C. *Int. J. Mass Spectrom.* **2003**, *227*, 191.

(43) Lagarrigue, M.; Bossée, A.; Afonso, C.; Fournier, F.; Bellier, B.; Tabet, J.-C. *J. Mass Spectrom.* **2006**, *41*, 1073.

(44) Lee, V. W.-M.; Li, H.; Lau, T.-C.; Siu, K. W. M. *J. Am. Chem. Soc.* **1998**, *120*, 7302.

(45) Payne, A. H.; Glish, G. L. *Int. J. Mass Spectrom.* **2001**, *204*, 47.

(46) Reiter, A.; Adams, J.; Zhao, H. *J. Am. Chem. Soc.* **1994**, *116*, 7827.

(47) Schmidt, A.-C.; Koppelt, J.; Neustadt, M.; Otto, M. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 153.

(48) Seto, C.; Stone, J. A. *Int. J. Mass Spectrom.* **1999**, *192*, 289.

(49) Shields, S. J.; Bluhm, B. K.; Russell, D. H. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 626.

(50) Vaisar, T.; Gatlin, C.; Rao, R. D.; Seymour, J. L.; Turecek, F. *J. Mass Spectrom.* **2001**, *36*, 306.

(51) Bluhm, B. K.; Shields, S. J.; Bayse, C. A.; Hall, M. B.; Russell, D. H. *Int. J. Mass Spectrom.* **2001**, *204*, 31.

(52) Chu, I. K.; Shoeib, T.; Guo, X.; Rodriquez, C. F.; Lau, T.-C.; Hopkinson, A. C.; Siu, M. K. W. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 163.

(53) Constantino, E.; Rimola, A.; Rodríguez-Santiago, L.; Sodupe, M. *New J. Chem.* **2005**, *29*, 1585.

(54) Dudev, T.; Lim, C. *Chem. Rev.* **2003**, *103*, 773.

(55) Parac, T. N.; Ullmann, G. M.; Kostic, N. M. *J. Am. Chem. Soc.* **1999**, *121*, 3127.

(56) Raffa, D. F.; Gómez-Balderas, R.; Brunelle, P.; Rickard, G. A.; Rauk, A. *J. Biol. Inorg. Chem.* **2005**, *10*, 887.

(57) Remko, M.; Rode, B. M. *Chem. Phys. Lett.* **2000**, *316*, 489.

(58) Remko, M.; Rode, B. M. *Struct. Chem.* **2004**, *15*, 223.

(59) Rulíšek, L.; Havlas, Z. *J. Phys. Chem. A* **2002**, *106*, 3855.

(60) Shoeib, T.; Rodríguez, C. F.; Siu, K. W. M.; Hopkinson, A. C. *Phys. Chem. Chem. Phys.* **2001**, *3*, 853.

(61) Xu, J. H.; Hu, C. W. *Acta Chim. Sin.* **2006**, *64*, 1622.

(62) Bush, M. F.; Forbes, M. W.; Jockusch, R. A.; Oomens, J.; Polfer, N. C.; Saykally, R. J.; Williams, E. R. *J. Phys. Chem. A* **2007**, *111*, 7753.

(63) Constantino, E.; Rodríguez-Santiago, L.; Sodupe, M.; Tortajada, J. *J. Phys. Chem. A* **2005**, *109*, 224.

(64) Rimola, A.; Rodríguez-Santiago, L.; Sodupe, M. *J. Phys. Chem. B* **2006**, *110*, 24189.

(65) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.

(66) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.

(67) Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623.

(68) Bauschlicher, C. W.; Ricca, A.; Partridge, H.; Langhoff, S. R. *Recent Advances in Density Functional Theory, Part II*; World Scientific: Singapore, 1997.

(69) Blomberg, M. R. A.; Siegbahn, P. E. M.; Svensson, M. *J. Chem. Phys.* **1996**, *104*, 9546.

(70) Holthausen, M. C.; Mohr, M.; Koch, W. *Chem. Phys. Lett.* **1995**, *240*, 245.

(71) Luna, A.; Alcamí, M.; Mó, O.; Yáñez, M. *Chem. Phys. Lett.* **2000**, *320*, 129.

(72) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372.

(73) Raghavachari, K.; Trucks, G. W.; Pople, J. A.; Head-Gordon, M. *Chem. Phys. Lett.* **1989**, *157*, 479.

(74) Wachters, A. J. *J. Chem. Phys.* **1970**, *52*, 1033.

(75) Hay, P. J. *J. Chem. Phys.* **1977**, *66*, 4377.

(76) Raghavachari, K.; Trucks, G. W. *J. Chem. Phys.* **1989**, *91*, 1062.

(77) McQuarrie, D. *Statistical Mechanics*; Harper & Row: New York, 1986.

(78) Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88*, 899.

(79) Weinhold, F.; Carpenter, J. E. *The Structure of Small Molecules and Ions*; Plenum: New York, 1988.

(80) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03, revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.

(81) Constantino, E. Unpublished work.

(82) Rodgers, M. T.; Armentrout, P. B. *J. Am. Chem. Soc.* **2002**, *124*, 2678.

(83) Cowan, J. A. *Inorganic Biochemistry: An Introduction*; VCH: New York, 1993.

(84) Saunders, M.; Houk, K. N.; Wu, Y. D.; Still, W. C.; Lipton, M.; Chang, G.; Guida, W. C. *J. Am. Chem. Soc.* **1990**, *112*, 1419.

(85) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765.

(86) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230.

(87) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

(88) *Probing of Proteins by Metal Ions and Their Low-Molecular-Weight Complexes*; Sigel, A., Sigel, H., Eds.; Metal Ions in Biological Systems 38; Marcel Dekker: New York, 2001.

(89) Corral, I.; Mó, O.; Yáñez, M. *J. Phys. Chem. A* **2003**, *107*, 1370.

(90) Corral, I.; Mó, O.; Yáñez, M. *New J. Chem.* **2003**, *27*, 1657.

(91) Corral, I.; Mó, O.; Yáñez, M. *Theor. Chem. Acc.* **2004**, *112*, 298.

(92) Corral, I.; Mó, O.; Yáñez, M. *Int. J. Mass Spectrom.* **2003**, *227*, 401.

(93) Scott, A. P.; Radom, L. *J. Phys. Chem.* **1996**, *100*, 16502.

(94) Poater, J.; Solà, M.; Rimola, A.; Rodríguez-Santiago, L.; Sodupe, M. *J. Phys. Chem. A* **2004**, *108*, 6072.

(95) Georgieva, I.; Trendafilova, N.; Rodríguez-Santiago, L.; Sodupe, M. *J. Phys. Chem. A* **2005**, *109*, 5668.

(96) Sodupe, M.; Bertran, J.; Rodríguez-Santiago, L.; Baerends, E. J. *J. Phys. Chem. A* **1999**, *103*, 166.

JP805764Y