Tandem Mass Spectrometric Evidence for the Involvement of a Lysine Basic Side Chain in the Coordination of Zn(II) Ion within a Zinc-bound Lysine Ternary Complex

Sunghyun Yu, Sunyoung Lee, Gyusung Chung,[†] and HanBin Oh[*]

Department of Chemistry, Sogang University, Seoul 121-742, Korea
[†]Department of Chemistry, Konyang University, Chungnam 320-711, Korea
Received June 21, 2004

We present the tandem mass spectrometry applications carried out to elucidate the coordination structure of Zn(II) bound lysine ternary complexes, $(Zn+Lys+Lys-H)^+$, which is a good model system to represent a simple (metallo)enzyme-substrate complex (ES). In particular, experimental efforts were focused on revealing the involvement of a lysine side chain ε-amino group in the coordination of Zn^{2+} divalent ions. MS/MS fragmentation pattern showed that all the oxygen species within a complex fell off in the form of H_2O in contrast to those of other ternary complexes containing amino acids with simple side chains (4-coordinate geometries, Figure 1a), suggesting that the lysine complexes have different coordination structures from the others. The participation of a lysine basic side chain in the coordination of Zn(II) was experimentally evidenced in MS/MS for Nε-Acetyl-L-Lys Zn(II) complexes with acetyl protection groups as well as in MS/MS for the ternary complexes with one NH_3 loss, $(Zn+Lys+Lys-NH_3-H)^+$. Detailed structures were predicted using *ab initio* calculations on $(Zn+Lys+Lys-H)^+$ isomers with 4-, 5-, and 6-coordinate structures. A zwitterionic 4-coordinate complex (Figure 7d) and a 5-coordinate structure with distorted bipyramidal geometry (Figure 7b) are found to be most plausible in terms of energy stability and compatibility with the experimental observations, respectively.

Key Words : Tandem mass spectrometry, Zinc ion, Lysine amino acid, Metal ion coordination, Binding structures

Introduction

Transition metal ions play a vital role in many catalytic reactions of metalloenzymes as well as in supporting structures of many known proteins.[1,2] Its active participation in many phenomena of the biological system is manifested in the finding that metalloenzymes compose approximately one third of known proteins. Transition metal ions often mediate the interactions of enzymes with its counterpart substrates by being involved in the formation of enzyme-substrate (ES) complexes. Interactions occurring around the metal ion can be recognized as metal-mediated ligand-ligand interactions, of which knowledge plays an important role in understanding the behaviors of chemical and biological phenomena related with ES complexes.[3-6] Intensive investigations have been made on metal-mediated ligand-ligand interactions and have revealed that these interactions can be categorized as hydrophilic, hydrophobic, or electrostatic, depending on their chemical, physical characteristics.[3-9] Furthermore, some intrinsic properties of ligands are often found to lead to modifications in their metal coordination structures. Although a significant progress has already been made in our understanding of the ES complexes, further advances in our knowledge are now retarded by difficulties arising from the complexity of ES complexes. Thus it has led one, including us, to investigate a simpler model system that can represent its structural and functional essence. Of particular interest to us is a ternary complex system composed of one transition metal ion and two constituent amino acid ligands, [M + A + B], where A and B are either the same or different amino acids.[4-6,8] This model system has been extensively used for more than two decades in the bio-inorganic and biophysical chemistry fields, so as to establish the perception that both ligand-ligand interactions and coordination structures are dependent very much upon identities of their side chains.
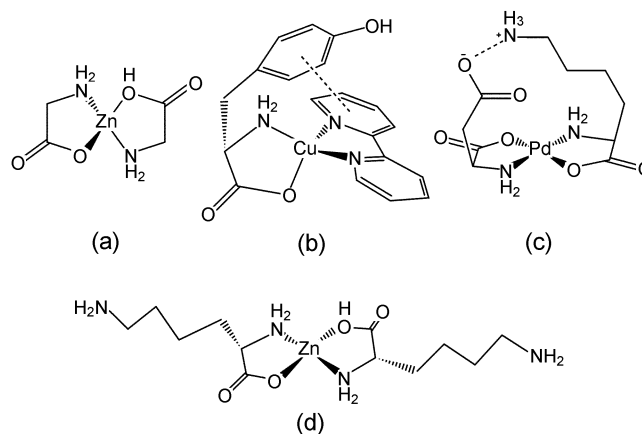
The metal-ion bound glycinate ternary complexes, (M +

Figure 1. Schematic structures of (a) $(Zn+Gly+Gly-H)^+$; (b) Cu(bpy)(L-tyrOH); (c) Pd(L-Asp)(L-Lys); (d) glycine complex-like $(Zn+Lys+Lys-H)^+$

[*]Corresponding Author. Tel: +82-2-705-8444; Fax:: +82-2-701-0967, e-mail: hanbinoh@sogang.ac.kr

Gly + Gly), in which a glycine ligand has a simple side chain of hydrogen, were shown to form a 4-coordinate structure. Whether it has a square planarity or tetrahedrality is known to be determined by the identity of a metal ion (Figure 1a). On the other hand, when a tyrosine or a phenylalanine ligand was involved, the aromatic ring was found to interact strongly with the ligand located across the metal ion, with its aromatic ring stacked over the other ligand.[10] Figure 1b displays a structure for a Cu(bpy)(L-tyrOH) complex proposed based on d-d transition CD (circular dichroism) spectral features corresponding to ligand-ligand charge transfer, where bpy stands for 2,2'-bipyridine.[6] In contrast to such a hydrophobic interaction, an electrostatic interaction between a protonated lysine side chain and a deprotonated aspartic side chain was reported for a Pd(L-Asp)(L-Lys) complex (see Figure 1c).[4] This interaction was deduced from CD spectral non-additivity of those of Pd(L-Asp)$_2$ and Pd(L-Lys)$_2$. However, in the case of Pd(L-Lys)$_2$, the ternary complex was shown to adopt a glycine complex-like structure in the acidic and weak basic conditions, owing to the repulsion between the positively charged Pd(II) ion and the protonated side chains of lysines. In general, the interactions of ligand-ligand or metal ion-ligand were found to rely in part on the polarity of solvent involved; for example, as the polarity of a solvent decreased, electrostatic interactions were found to become more important.[4] Electrostatic interactions are far more important in the biological systems located within hydrophobic environments, such as in the active sites of enzymes, and therefore are regarded as a main driving force to appropriately orient molecular species in the formation of enzyme-substrate complexes and also in the subsequent reactions.

Here, light is shed on polar interactions within a gas-phase Zn(II) bound lysine ternary complex, Zn(L-Lys)$_2$, in which basic ε-amino side chains are expected to remain neutral in opposite to previous solution-phase Pd(L-Lys)$_2$ studies in which the side chains of lysine ligands were positively charged through protonation.[4] This model system is expected to well represent polar interactions that play an essential role in Zn-containing metalloenzyme-substrate reactions. The model system was readily prepared in the gas phase using electrospray ionization (ESI), and was subjected to ample investigations utilizing MS^n tandem mass spectrometry techniques and chemical variations. A gas phase study adopted here has a unique advantage of exploring intrinsic properties of polar interactions within a ternary complex, without any complicated solvent effect.[11] In general, mass spectrometric analyses exclude any interference that may arise from impurities or unwanted mixture complexes which is extremely difficult to separate in other spectroscopic studies. Zn(II) ion is chosen here as a metal ion that acts as a Lewis acid in the catalytic reactions of *Carbonic anhydrase, carboxypeptidase A, alcohol dehdrogenase*, etc.[1,212-16] Detailed mass spectrometric understanding has already been made for Zn bound amino acid binary systems, (A.A. + Zn H)$^+$ by Ohanessian *et al.* and thus are expected to provide a sound foundation on to interpret the MS/MS data aimed for

fine understanding of ligand-ligand or metal-ligand interactions and their corresponding structures.[17-20] In addition, *ab initio* calculations will present some possible gas-phase structures with some specific coordination numbers.

Experimental Section

Experiments were performed on a commercial 4.7 Tesla Fouier-transform mass spectrometer (Ionspec, Inc., Lake Forest, CA) equipped with electrospray capabilities. Spray solutions were prepared by dissolving 0.1 mM L-amino acids of interest and 0.1 mM Zn(OAc)$_2$ in 50 : 50 (v : v) water:acetonitrile solutions. Electrospray was achieved by eluting spray solutions at 1-2 μL/min rates, using a syringe infusion pump (Harvard apparatus, model 22, Holliston, MA). A stainless tube or a home-pulled fused silica capillary with 100 μm I.D. was used for ESI. A 3.2-3.5 kV potential was applied to a zero-volume union through which spray solutions flowed. As a drying gas, nitrogen gas heated to 200 ^{o}C was used. Ionized molecules were externally accumulated for 2 seconds in a hexapole trap before being pulsed into a long single stage quadrupole guide leading to an ion cyclotron resonance (ICR) cell. Trapping of the ions in the ICR cell was made using both gated-trapping and collision gas cooling methods.

For MS/MS tandem mass spectrometry studies, the ions of interest were isolated using one broadband SWIFT (Stored Waveform Inverse Fouier Transform) waveform.[21] By setting the SWIFT mass isolation widow width to ~0.75 Th, all the other ions were completely removed. The isolated ions were then subjected to +1.0 kHz off-resonance SORI-CAD (Sustained Off-Resonance Irradiation Collisional Activation Dissociation), and therein collision energies were carefully controlled so as to induce at least 70% fragmentations. For this, ~40 mTorr nitrogen collision gas was introduced for ~25 ms by an incorporated pulsed valve (General Valve, Fairfield, NJ).[22] For MS^n ($n \geq 3$) studies, the procedures described above were repeated multiple times, with a longer delay time before detection, necessary for maintenance of low pressure (~10^{-9} torr) at the cell.

Triply distilled water was used as an ESI solvent, and chemicals including amino acids and HPLC-grade acetonitrile solvents (Sigma Korea, Seoul) were used without further purification.

The molecular structures of possible (Zn+Lys+Lys−H)$^+$ isomers were fully optimized at the RHF levels with the SBJKC effective core potential.[23,24] The RHF/SBKJC hessians (matrix of energy second derivatives) were calculated in order to confirm whether or not the stationary points are indeed local minima. A reliable energy comparison was also sought by performing the MP2 single point calculations.[25] All the calculations were executed using GAMESS program.[26]

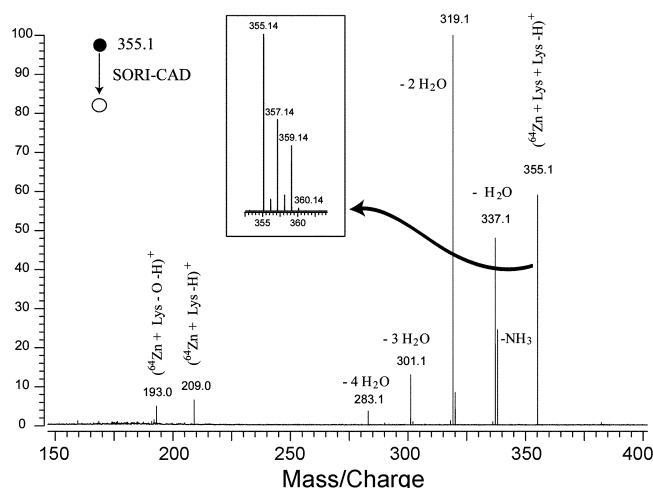## Results and Discussion

Covalently bound (Zn+Lys+Lys−H)$^+$ complexes. Here,

Figure 2. SORI-CAD MS/MS spectrum on $(^{64}Zn+Lys+Lys-H)^+$; '–' symbol here indicates deprotonation. Identifications for fragment ions are given above their corresponding mass spectral peaks. Inset: enlarged isotopic cluster distribution of $(Zn+Lys+Lys-H)^+$.

MS/MS tandem mass spectrometry approaches were adopted for singly charged deprotonated Zn(II) bound L-lysine ternary complex ions, $(Zn+Lys+Lys-H)^+$, with an aim to obtain structural information of this ternary complex. The complexes were prepared by electrospraying a mixture solution of 0.1 mM $Zn(OAc)_2$ and 0.1 mM L-lysine. The resulting molecular-ion mass spectrum exhibited complicated mass spectral features, containing a variety of Zn(II), lysine, acetate mixture complexes (spectrum not shown). However, the Zn(II) bound lysine ternary complex ions could be easily distinguished from other complexes by its unique isotopic distribution. Specifically, Zn isotopes have 64, 66, 67, and 68 Da masses with their relative natural abundances $100 : 57 : 8 : 39$, respectively, and thus Zn(II) bound ternary complexes display a unique isotopic distribution as shown in the inset of Figure 2.

Among isotopic clusters, the most abundant isotope of $(^{64}Zn+Lys+Lys-H)^+$ was isolated by one broadband SWIFT waveform and then was subjected to SORI-CAD, resulting in fragmentation mass spectrum of Figure 2. To our surprise, the ions corresponding to abnormal, extensive H_2O losses (up to four losses) were observed as major products along with those of a NH_3 loss or of Zn(II) bound lysine binary complexes, $(^{64}Zn+Lys-H)^+$. In order to verify whether respective ions contain a Zn atom, the identical experiment was also performed on the second most abundant isotope complexes of $(^{66}Zn+Lys+Lys-H)^+$, and it confirmed our mass spectral interpretation. This unique, abnormal fragmentation behavior was in sharp contrast to that observed in the SORI-CAD on proton-bound lysine dimers of $(Lys+Lys+H)^+$ in which monomeric dissociation into $(Lys+H)^+$ was dominant (data not shown). This can be understood in terms of the covalent bonding character of Zn(II) bound ternary complexes as opposed to proton bound dimers with a noncovalent bonding character.[4-9] In the covalently bound ternary system, given collision energy resulted dominantly

in loss of labile H_2O or NH_3 rather than in that of the noncovalent monomer unit. The observation that internal covalent fragmentations were prevalent over noncovalent monomeric dissociations is consistent with those of previously reported CAD studies on the other transition metal bound complexes.[10]

Abnormal Extensive H_2O losses in $(Zn+Lys+Lys-H)^+$. Most notable in Figure 2 is the observation that H_2O losses, occurring in general through rearrangement reactions, took place extensively up to the point that all the oxygen species fell off as a H_2O. Even though H_2O losses are commonly observed in the CAD studies for peptides or proteins, complete oxygen fall-offs in the form of a H_2O loss, as shown in this case, have never been reported previously. Exceptionally, in the case of proteins, *Carbonic Anhydrase* containing one Zn(II) ion were previously shown to exhibit extensive H_2O losses rather than backbone dissociations in high temperature BIRD (Blakbody Infrared Radition Dissociation) experiments.[27] Two initial H_2O losses are expected to originate from two $-OHs$ on the carboxyl functional groups [*e.g.*, C(O)-OH], in which proton transfer onto the $-OH$ is likely to make the resulting C(O)-OH_2^+ susceptible to H_2O loss. Two extra H_2O losses might occur *via* reduction of carbonyl oxygens by some intriguing mechanism. It is expected that there may exist a variety of plausible pathways leading to extensive H_2O losses, but the scrutiny of all these pathways is, at this current stage of investigations, beyond the scope of this report.

In order to verify whether these H_2O losses took place in sequence or not, daughter complexes with one or two H_2O losses were re-isolated and were subjected to the second SORI-CAD. The resulting MS/MS/MS spectrum of Figure 3 shows that sufficient collision energies given to the daughter ions induced additional H_2O losses. It also manifests that in this lysine ligated system, reaction pathways leading to H_2O losses generally have lower activation-energy barriers than the ones to monomeric dissociation or to other type of internal fragmentations.

We sought the identical MS/MS experiments on other ternary complexes with different representative amino acids for the purpose of verifying whether extensive H_2O losses
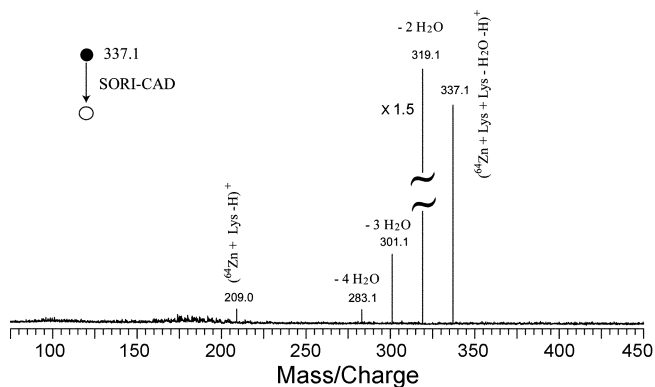

Figure 3. MS/MS/MS spectrum obtained by SORI-CAD on $(^{64}Zn+Lys+Lys-H_2O-H)^+$.

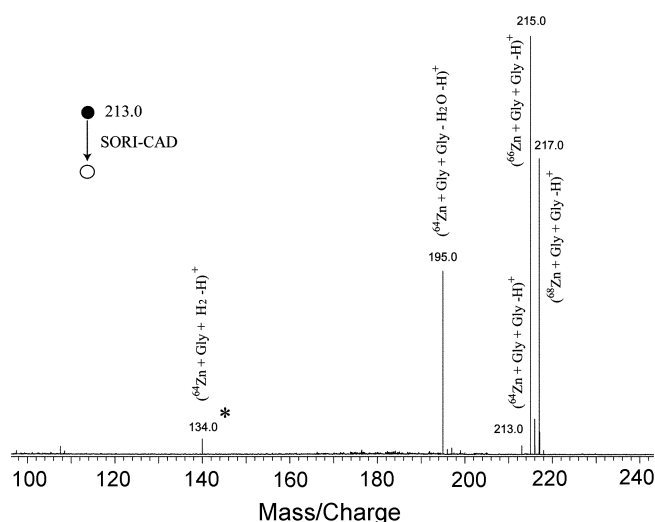**Figure 4**. SORI-CAD MS/MS spectrum on (Zn+Gly+Gly–H)$^+$. SORI-CAD was performed only on ^{64}Zn isotopic complexes, even though here the whole isotopic clusters of (Zn+Gly+Gly–H)$^+$ were isolated in contrast to other experiments. *Note that a H$_2$ molecule is adducted onto a monomeric complex.

occur in other ternary systems as well. For a glycine ternary system (Zn+Gly+Gly–H)$^+$, main fragment ions were the ones with one H$_2$O loss, but further H$_2$O loss was not observed (see Figure 4). This result is also quite distinct from that of previous MS/MS studies on glycine binary complex, (Zn+Gly–H)$^+$, in which MS/MS yielded mainly CO$_2$ loss or CO + H$_2$O losses. It indicates that the glycine ternary complexes have a quite different chemical bonding character from that of the binary complexes.[17-20] For the other ternary complexes having amino acids with non-basic side chains like (Zn+Ala+Ala–H)$^+$ or (Zn+Ile+Ile–H)$^+$, SORI-CAD resulted in fragmentation spectra (spectra not shown) similar to that of the glycine ternary complexes. Since a change in the coordination environment is expected to induce variation in the fragmentation behavior, these observations imply that the basic side chain of a lysine may participate, directly or indirectly, in Zn(II) coordination. If lysine side chains would stretch outward from the coordination center as in Figure 1d, MS/MS patterns of these complexes should not differ much from that of glycine complexes (refer to Figure 1a). This postulation can be further supported by the fact that a nitrogen atom with strong basicity would have a reasonable tendency to coordinate a strong Lewis acid of Zn(II).[28]

Involvement of the Lys side chain in the coordination of Zn(II) ions. SORI-CAD was performed on Nε-Acetyl-L-Lys complexes in which basic ε-amino side chains were protected by rather bulky acetyl groups (see Figure 5a). The resulting fragmentation spectrum in Figure 6 displays that H$_2$O loss takes place only up to two losses and that instead both proton bound monomer unit (Nε-Acetyl-Lys+H)$^+$ and Zn(II) bound binary complex (Zn+Nε-Acetyl-Lys–H)$^+$ appear with significant abundances. Weaker binding of Nε-Acetyl-Lys to Zn(II) appears to lead to monomeric dissociation upon SORI-CAD, and it provides information related
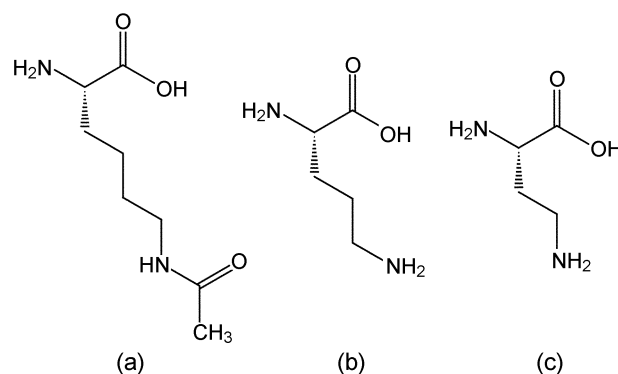

Figure 5. Structural formulae of (a) Nε-Acetyl-L-Lysine; (b) L-Ornithine; (c) (S)-(+)-2,4-Diaminobutyric acid.

with structures of normal lysine ternary complexes. If lysine ternary isomers would have their side chains extending outward as in Figure 1d, their MS/MS fragmentation pattern would not show any change when their basic ε-amino side chains were blocked by acetyl groups. These observations exhibit strong supporting evidences that a basic ε-amino side chain is involved in coordination of Zn(II) ion. Another interesting observation to note here is that proton is rather free to move around within a complex, as shown in the formation of proton bound lysine monomer, (Nε-Acetyl-Lys+H)$^+$ (*vide infra*).

The influence of the length of a lysine ε-amino side chain on the MS/MS fragmentation pattern was explored by applying SORI-CAD to L-Ornithine and (S)-(+)-2,4-Diaminobutyric acid complexes whose side chains have one or two -CH$_2$- units shorter, respectively, than the lysine side chain (refer to Figure 5b and 5c). The fragmentation spectra for lysine homologue ternary complexes revealed that H$_2$O
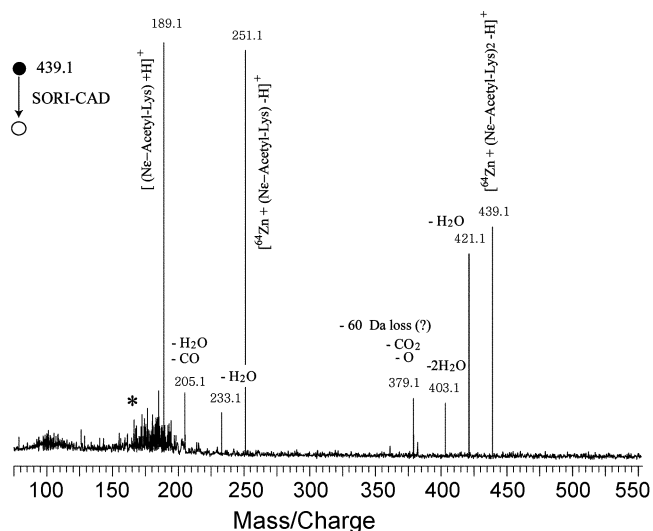

Figure 6. SORI-CAD MS/MS spectrum on [^{64}Zn+(Nε-Acetyl-Lys)$_2$–H]$^+$. Proton-bound monomers [(Nε-Acetyl-Lys)+H]$^+$ as well as Zn-bound complexes [^{64}Zn+(Nε-Acetyl-Lys)–H]$^+$ are major fragment products. *Noises appearing in the mass range of *m/z* 90 to 220 are a left-over peak from previous experiments for synthetic polymer analysis, and thus should be ignored in the mass spectral interpretation.
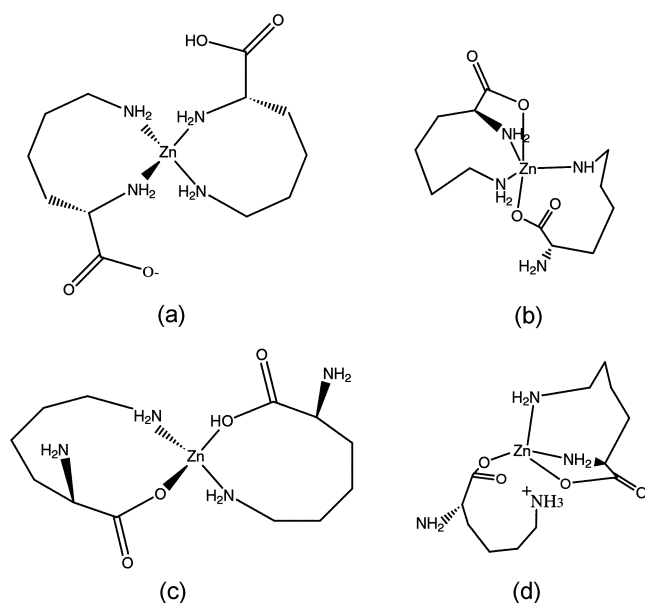
Figure 7. Skeletal structural formulae of $(Zn+Lys+Lys-H)^+$, obtained by *ab initio* calculations: (a) 4 coordinate structure with two 8-membered rings; (b) 5 coordinate structure; (c) 4 coordinate structure with two 9-membered rings; (d) zwitterionic 4 coordinate structure.

Figure 8. MS/MS/MS spectrum obtained by SORI-CAD on $(^{64}Zn+Lys+Lys-NH_3-H)^+$.

losses were still major fragment ions, but that H_2O losses occurred only up to three and Zn(II) bound binary complex (fragmentation product) did not show up at all, suggesting the stronger amino-acid binding to Zn(II). Stronger coordination by the lysine homologues was also evidenced in the relative abundances of NH_3 losses in their MS/MS spectra. Interestingly, NH_3 loss, which was half as abundant as H_2O loss in the case of lysine complexes, took place only quarterly for Ornithine and not at all for (S)-(+)-2,4-Diaminobutyric acid complexes (spectra not shown). Zn(II) coordination by a lysine ε-amino group requires the formation of a 8 or 9-membered ring that would exert significant angle strain. This severe angle strain in the lysine complexes is expected to be relieved in Ornithine (7 or 8-membered ring) or (S)-(+)-2,4-Diaminobutyric acid (6 or 7-membered ring) complexes, and thus coordination binding strengthen accordingly (see Figure 7).[5] To summarize, variation in coordination strengths for lysine and its homologue complexes provided another evidence for ε-amino group participation in Zn coordination.

Strong N-binding in $(Zn+Lys+Lys-H)^+$. Knowledge of how NH_3 or H_2O losses are related with Zn(II) coordination by lysines was deepened by further examining them using SORI-CAD. As indicated in Figure 8, daughter complexes with one NH_3 loss undergo monomeric dissociation to $(Zn+Lys-H)^+$ as well as produce H_2O losses upon SORI-CAD application, with the two fragmentation-channels being of relatively comparable abundances. This was in sharp contrast to the case where the daughter complexes with H_2O losses mainly exhibited additional H_2O losses (Figure 3). Once stronger nitrogen binding to Zn(II) ion disappeared as a result of NH_3 loss, the resulting daughter complexes t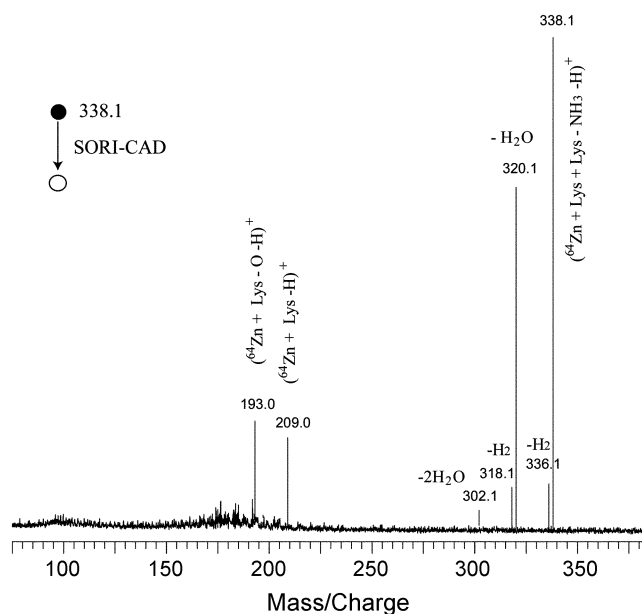ended to lose the lysine moiety with one NH_3 loss. The stronger nitrogen binding to Zn(II) over oxygen binding is consistent with "Pearsons principle"; '*Hard acids prefer to bind to hard bases and vice versa*'. In general, Zn(II) with a small radius is regarded as a hard Lewis acid, and therefore tends to prefer nitrogen of higher basicity to oxygen.[28] This principle was also confirmed in a separate experiment in which Ca^{2+} lysine ternary complexes, (Ca+Lys+Lys−H)^+, produced much more abundant monomeric dissociation products, (Ca+Lys−H)^+, at the application of SORI-CAD (in general, Zn^{2+} is known to be a harder Lewis acid than Ca^{2+}) [data not shown].

The observed NH_3 loss could originate from two possible sources, α-amino or ε-amino group. Related with this, ternary complexes with simple side chains like Gly [H], Ala [CH_3], and Ile [$CH(CH_3)CH_2CH_3$], whose structures are expected to be all similar to the one in Figure 1a, did not yield any noticeable NH_3 loss, suggesting that NH_3 was probably lost from the ε-amino group in the side chain.

In addition, H_2 loss was also observed in Figure 8, SORI-CAD fragmentation spectrum for $(Zn+Lys+Lys-NH_3-H)^+$; *e.g.*, $(Zn+Lys+Lys-NH_3-H_2-H)^+$ and $(Zn+Lys+Lys-NH_3-H_2O-H)^+$. This can also be related with the observation of an oxygen loss in the Zn(II) bound binary complexes $(Zn+Lys-O-H)^+$ (see Figure 8). A similar H_2 loss was also previously reported in CAD studies for Ni(II) bound dipeptides, where authors suggested the existence of isomers with two hydrides, H^-, coordinating Ni(II) cation.[29] These findings confirm that hydrogen species such as H^+ and H^- are rather free to migrate within a metal ion-bound complex, even across the metal ion, as shown in the formation of proton-bound lysine monomer, $(N\varepsilon-Acetyl-Lys+H)^+$, upon SORI-CAD application to $N\varepsilon$-Acetyl-L-Lys Zn(II) complexes, consistent with Wysockis mobile proton model.[30]

Proposed structures of bis-lysated zinc complex, (Zn+Lys+Lys−H)⁺. Represented above were experimental evidence that the ε-amino group of the lysine side chain is involved in the coordination of Zn(II) cation. Then what structures will the Zn(II) bound lysine ternary complexes take? Even though above tandem mass spectrometric studies could not clearly dictate a specific structure, a few candidate structures, supported by *ab initio* calculations, can be suggested. Here the extent of calculations is narrowed down, based on the experimental observations and chemical insight, to plausible complex structures rather than pursuing exhaustive full computational search.

Zn(II) cation with d^{10} electronic configuration, in general, favors a tetrahedral structure, but 5- or 6-coordinate structures are possible as well. Since there are three possible metal chelating sites in a lysine molecule, one can expect several possible 4-, 5-, 6-coordinate isomers of (Zn+Lys+Lys−H)⁺. When only the carboxy oxygen and α-amino group are chelated to Zn(II), the simple 4-coordinate isomer is formed with two 5-membered rings, whose structure is close to that of Figure 1d. In the cases where lysine side chain(s) participates in Zn(II) coordination, Zn complexes can adopt not only a 4-coordinate structure, but also 5-, 6-coordinate structures, depending on the number of ligand side chains involved in the chelation to Zn(II) ion (see Figure 7; 6-coordinate structure not shown). In particular, a 4-coordination structure with two 9-membered rings, in which only the carboxy oxygens and ε-amino groups are chelated to Zn(II) (Figure 7c), is expected to be quite unstable due to its significant angle strain.

Initially, calculations were carried out on the isomers with the deprotonation site fixed only at one carboxy acid; the zwitterions isomers with two deprotonated carboxy acids and one protonated ε-amino group will be considered later in the text. Intensive geometry optimizations showed that there was no local minimum point corresponding to 6-coordinate complex (structure not shown), whereas several stationary structures with 4- or 5-coordination were found (Figure 7a, b). The MP2/SBKJC calculation estimated that the 5-coordinate structure with each two lysine chelating Zn(II) tridentately and bidentately, respectively, is the most stable isomer (Figure 7b). In this structure, three amino groups of two lysine molecules are located on the equatorial positions, while the two oxygen atoms are on the axial positions. Overall feature of the 5-coordinate structure is predicted to be distorted trigonal bipyramidal geometry. On the other hand, two types of 4-coordinate complexes are found on the energy hypersurface (Figure 1d and 7a). The MP2 calculation predicts that the 4-coordinate complexe with two 5-membered rings is about 40 kcal/mol higher in energy than the 5-coordinate one. The overall geometry of this 4-coordinate complex appears to be somewhat distorted tetrahedral structure. Among three possible 4-coordinate structures (Figure 1d, Figure 7a, c), the one with two 8-membered rings (Figure 7a) is predicted to be more stable by 14 kcal/mol than the one with two 5-membered rings (Figure 1d), despite the unfavorable angle strain. This suggests that

the ligating ability plays a more important role in deciding the stability of the complexes than the ring strain does.

Zwitterion complexes, in which the proton of a carboxy acid is transferred to the ε-amino group in the same lysine molecule, were also considered through *ab initio* calculation. The most stable structure was found to be a 4-coordinate complex (Figure 7d) with its energy being ~20 kcal/mol lower than the stable 5 coordinate complex (Figure 7b). In this complex, the protonated ε-amino group does not coordinate the Zn(II) ion, and instead it is located closely to the negatively charged deprotonated carboxy oxygen. This finding is in accord with that of Williams' in which alkali-metal ion bound complexes favor the zwitterions structure.[31] This zwitterionic form is stabilized by the presence of Zn(II) ion and is also strengthened by the high proton affinity of a lysine ε-amino group, consistent with Williams' explanation for the factors affecting the stability of zwitterions. Although this zwitterionic complex is found to be most stable, in particular, of lower energy than the trigonal bipyramidal complex, this structure does not fully support the experimental finding that the monomeric dissociations upon SORI-CAD occur as a minor pathway. A close look at the structure indicates that the zwitterion lysine molecule is bonded to the Zn(II) in a monodentative manner, thus rendering this molecule susceptible to detachment from the Zn(II) upon the application of SORI-CAD (monomeric dissociation). On the contrary, the trigonal bipyramidal complex suggests a stronger lysine binding to Zn(II); bidentation vs monodentation. For reference, in the previous studies for (Zn+Gly−H)⁺ complex it was found that the molecular ions detected in ESI-MS did not reflect the energy stability due to the 'kinetic trapping' occurring during the ESI solvent evaporation process.[17-20] Despite such a conflict, in both structures the lysine side chain was, however, found to be involved in the coordination of Zn(II), consistent with experimental observations of extensive H_2O losses.

In the structures supported by tandem mass spectrometric or above computational studies, two lysine ligands interact indirectly through the mediation of Zn(II) cation. This is quite different from the case of Cu(bpy)(L-tyrOH) complexes where the aromatic ring of tyrosine was shown to directly interact with the bpy oxygen (hydrophobic interaction), and also from the case of Pd(L-Asp)(L-Lys) where a positively protonated side chain and a negatively deprotonated side chain strongly interact *via* electrostatic attraction.[4-6] The suggested structures are also in sharp contrast to the one of glycinated complexes in that the side chains of lysine complexes are actively participating in Zn(II) coordination.

To summarize, when Zn(II), a strong Lewis acid, is coordinated by two homo amino acids with basic side chains, it is likely that it forms a zwitterionic 4-coordinate complex or a 5-coordinate structure with a basic side chain chelating Zn(II). However, authors want to point out that it is too premature to generalize this observation to other systems with multi-dentate basic sites. Generalization of our conclusions would require further extensive and rigorous investigations.

Conclusions

A metal cation bound amino acid ternary complex is a good model system to provide structural and functional understanding of an enzyme-substrate complex which is an essential part in metalloenzyme catalytic reactions. Here, Zn(II) bis-lysinate complexes, (Zn+Lys+Lys–H)$^+$, were synthesized *in vacuo* using an electrospray ionization method, and its structural characteristics were explored in a detailed manner *via* SORI-CAD tandem mass spectrometry. Fragmentation patterns obtained in MS^n approaches for lysine and its analogue complexes provided ample evidence that the basic side chain of a lysine ligand is actively involved in the coordination of Zn divalent cation. It contrasts with previous CD, NMR studies for Pd(L-Asp)(L-Lys) in which a protonated lysine side chain was shown to directly interact in an electrostatic manner with other deprotonated ligand across the metal ion.[4-6] Related with this, supporting calculations suggested that a zwitterionic 4-coordinate complex (Figure 7d) is most stable in energy, and that a 5- coordinate complex structure of Figure 7b is more stable than the 4 or 6-coordinate structures (Figure 1d, 7a/c) and supports well the experimental findings. Elucidation of more detailed structural characteristics is now in progress, including stable isotope labeling experiments in which nitrogens in ε-amino groups are labeled to ^{15}N, and an infrared spectroscopic approach which is expected to distinguish the bonding character of many −OH or −NH moiety in the complexes. As this report has shown, mass spectrometry is a promising tool to provide the structural information of biomolecules in the gas-phase as well as to analyze biomolecules, in particular, when coupled with various chromatography methods.[32]

References

1. Bertini, I.; Gray, H. B.; Lippard, S. J.; Valentine, J. S. *Bioinorganic Chemistry*; University Science Books: New York, 1994.
2. Lippard, S. J.; Berg, J. M. *Principles of Bioorganic Chemistry*; University Science Books: New York, 1994.
3. Mildvan, A. S. In *The Enzymes*, 3rd ed.; Boer, P. D., Ed.; Academic Press: New York, 1970; Vol. II, p 445.
4. Yamauchi, O.; Odani, A. *J. Am. Chem. Soc.* **1981**, *103*, 391.
5. Sigel, H.; Martin, B. R. *Chem. Rev.* **1982**, *82*, 385, and references therein.
6. Yamauchi, O.; Odani, A. *J. Am. Chem. Soc.* **1985**, *107*, 5938.
7. Sigel, H. In *Advances in Solution Chemistry*; Bertini, I.; Lunazzi, L.; Dei, A., Eds.; Plenum: New York, 1981; pp 149-159.
8. Fischer, B. E.; Sigel, H. *J. Am. Chem. Soc.* **1980**, *102*, 2998.
9. Malini-Balakrishnan, R.; Scheller, K. H.; Haring, U. K.; Tribolet, R.; Sigel, H. *Inorg. Chem.* **1985**, *24*, 2067.
10. Hu, P.; Sorensen, C.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 1079, and references therein.
11. Oh, H.-B.; Breuker, K.; Sze, S. K.; Ying, G.; Carpenter, B. K.; McLafferty, F. W. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15863.
12. Christianson, D. W. *Adv. Protein Chem.* **1991**, *42*, 281.
13. Berg, J. M.; Godwin, H. A. *Annu. Rev. Biophys. Biomol. Struct.* **1997**, *26*, 357.
14. Berg, J. M.; Shi, Y. *Science* **2003**, *300*, 1081.
15. Ashagiri, D.; Pratt, L. R.; Paulaitis, M. E.; Rempe, S. B. *J. Am. Chem. Soc.* **2004**, *126*, 1285.
16. Lin, Y.-L.; Lim, C. *J. Am. Chem. Soc.* **2004**, *126*, 2602.
17. Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. *Int. J. Mass Spectrom.* **2000**, *201*, 307.
18. Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. *Int. J. Mass Spectrom.* **2001**, *204*, 267.
19. Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. *Int. J. Mass Spectrom.* **2001**, *206*, 45.
20. Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. *Int. J. Mass Spectrom.* **2003**, *227*, 439.
21. Marshall, A. G.; Wang, T. C. L.; Ricca, T. L. *J. Am. Chem. Soc.* **1985**, *107*, 7893.
22. Gauthier, J. W.; Trautman, T. R.; Jacobson, D. B. *Anal. Chim. Acta* **1991**, *246*, 211.
23. Stevens, W. J.; Basch, H.; Krauss, M. *J. Chem. Phys.* **1984**, *81*, 6026.
24. Stevens, W. J.; Basch, H.; Jasien, P. *Can. J. Chem.* **1992**, *70*, 612.
25. Pople, J. A.; Binkley, J. S.; Seeger, R. *Int. J. Quantum Chem.* **1976**, *S10*, 1.
26. Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, K.; Matsunaga, N.; Nguyen, K. A.; Su, S.; Windus, T. L.; Dupuis, M.; Montgomery, J. A. *J. Comput. Chem.* **1993**, *14*, 1347.
27. Ge, Y.; Horn, D. M.; McLafferty, F. W. *Int. J. Mass Spectrom.* **2001**, *210/211*, 203.
28. Huheey, J. E.; Keiter, E. A.; Keiter, R. L. *Inorganic Chemistry : Principles of Structure and Reactivity*, 4th ed.; Addison Wesley Pub. Co.: Harlow, 1997.
29. Reiter, A.; Adams, J.; Zhao, H. *J. Am. Chem. Soc.* **1994**, *116*, 7827.
30. Dongré, A. R.; Jones, J. L.; Somogyi, Á.; Wysocki, V. H. *J. Am. Chem. Soc.* **1996**, *118*, 8365.
31. Lemoff, A. S.; Bush, M. F.; Williams, E. R. *J. Am. Chem. Soc.* **2003**, *125*, 13576, and references therein.
32. Yoon, K. H.; Kim, W.; Park, J. S.; Kim, H. J. *Bull. Korean Chem. Soc.* **2004**, *25*, 878.