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Structures and fragmentations of zinc(II) complexes of amino acids in the gas phase. III. Rearrangement versus desolvation in the electrospray formation of the glycine-zinc complex

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Abstract

The zinc complex of deprotonated glycine (Gly), denoted $[Gly-H+Zn]^+$, is readily formed in the gas phase by electrospray ionization. Low energy collisional activation of $[Gly-H+Zn]^+$ leads to three primary fragments, resulting from the losses of CO_2, H_2O+CO, and CO. Previous work has shown that the first two reactions require isomerization of the glycinate to nonclassical structures before the last desolvation step, and that loss of CO can only occur from a N-deprotonated glycine complex. It is shown herein, using accurate ab initio calculations, that such a structure does not pre-exist in solution, and that it is also formed in the electrospray process, during one of the last desolvation steps. Solvent molecules participate in this mechanism as proton relays between the two functional groups of Gly. These results provide a complete picture of the fragmentation of gaseous $[Gly-H+Zn]^+$: each of the primary fragmentations arises from a specific precursor, none of which is the parent structure formed in solution. (Int J Mass Spectrom 206 (2001) 45–52) © 2001 Elsevier Science B.V.

Keywords: Electrospray; Desolvation; Glycine–zinc complex; Mechanisms; Ab initio calculations

1. Introduction

Despite the biological importance of zinc, there have been comparatively few studies of its complexes with biomolecules in the gas phase. Metal–peptide and metal–protein interactions have been investigated to gain structural information on metal-induced conformational changes as indicated by the observed charge distributions [1–4]. Accurate mass measurements were shown to reveal the extent of thiol ligand deprotonation [5]. Collisional activation experiments on zincated histidine-containing peptides indicated fragmentation at histidine sites, suggesting zinc ion binding primarily to histidine [6,7].

We have undertaken a study of the structures and fragmentation modes of the gas-phase complexes of zinc attached to deprotonated amino acids $[AA-H+Zn]^+$, as formed when an amino acid (AA) and $ZnCl_2$ are dissolved in a water/methanol solution [8,9]. Such species are expected to exist as carboxylate complexes because deprotonation occurs at the carboxylic function, which is clearly the most acidic (see **1** on Fig. 1). In the previous article of this series [9], we have shown that there exist three primary fragmentations of low energy for $[Gly-H+Zn]^+$: loss of CO_2, of H_2O+CO and of CO. Loss of H_2O+CO was shown to occur by sequential loss of

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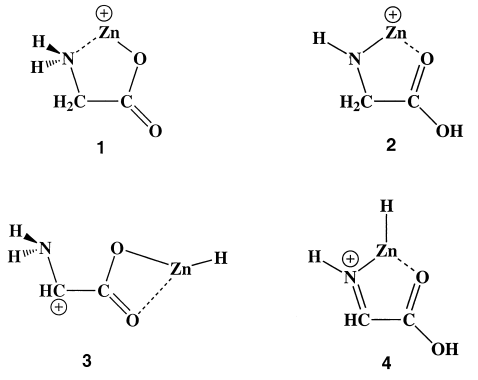

Fig. 1. Most stable isomers of $[Gly-H+Zn]^+$.

H_2O first, followed by elimination of CO, therefore the three fragmentations are true parallel pathways. In order to understand these fragmentations, and to establish a possible relationship between the mode of metal attachment to the amino acid and the fragmentations observed, a comprehensive mechanistic study was presented by means of ab initio calculations.

An unexpected result of these calculations is that **1** cannot give rise to any of the observed fragment ions. Indeed, the lowest energy path from **1** is loss of CO_2+Zn to yield the immonium ion $CH_2NH_2^+$, a fragment that is never observed. This rules out the hypothesis that under collision conditions, **1** would isomerize to some other structure before fragmentation. This led us to investigate the occurrence of isomerization during the electrospray process. We showed [8] that within the last precursors of $[Gly-H+Zn]^+$, which are [**1**,CH_3OH] (major) and [**1**,Gly] (minor), isomerization of **1** into **3** (Fig. 1) requires less energy than does elimination of CH_3OH or Gly. This isomerization occurs by β-H transfer, a well known elementary process of organometallic complexes in both gas and solution phase. The new, nonclassical structure **3** can itself isomerize into **4** (Fig. 1), either before or after final solvent evaporation. It was shown that **3** and **4** are the precursors for the losses of CO_2 and H_2O+CO, respectively.

How does the observed loss of CO occur? It has been shown [9] that the appropriate precursor for this fragmentation is **2** (Fig. 1), but its mode of formation remains unexplained. Our previous work has shown that direct interconversion between gaseous **1** and **2** can be ruled out on energetic grounds. Two hypotheses can then be formulated: either **2** is formed in solution together with **1**, or else it is formed at some point in the gas phase. In this article, we study the complexes of **1** and **2** with one methanol molecule and with one through four water molecules. The results show that **1** is, as previously assumed, the only structure formed in solution. **2** is formed in the precursor ion $[CH_3OH+Gly-H+Zn]^+$ by proton transfer between the amino and carboxylate ends of Gly. Although this is unfavorable from **1**, it becomes relatively easy in the precursor because the methanol molecule can play the role of proton relay. These results provide another mechanism for ion isomerization during electrospray ionization. Together with our previous results, they provide a complete picture of the formation, structures and fragmentations of $[Gly-H+Zn]^+$ in the gas phase.

2. Computational

As in our previous work [8,9], two basis sets were used in this study. For geometry optimizations and vibrational frequency calculations, the 6-31G* basis was used for H, C, N, and O, and the Wachters [14*s*9*p*5*d*1*f*/9*s*5*p*3*d*1*f*] basis was used for Zn [10]. This is referred to as basis1. For final energy calculations, basis2 consists in the 6-311+G(2*d*,2*p*) basis for H, C, N and O, and the extended Wachters basis [15*s*11*p*6*d*2*f*/10*s*7*p*4*d*2*f*] for Zn. Geometry optimizations and vibrational frequency calculations were carried out at the HF/basis1 level. These calculations were also used to obtain zero point vibrational energies and thermal corrections at 298 K (E_{therm}). Extensive tests [8,9] showed that the use of HF rather than second order Møller-Plesset (MP2) geometries leads to very small errors in final energy calculations. Final energetics were obtained with MP2(FC)/basis2 wave functions at the HF/basis1 geometries, a level denoted below as MP2(FC)/basis2//HF/basis1 (where FC indicates that the frozen core approximation was applied to the 1*s* electrons of C, N, and O and to the 1*s*,

2*s* and 2*p* electrons of Zn). The GAUSSIAN98 program package [11] was used throughout.

3. Results

3.1. Structure of solvated [Gly−H+Zn]$^+$

The structure of the zinc–glycinate moiety has been studied by x-ray diffraction in both solid [12] and liquid [13] media. Although all discussions assume that glycine is O deprotonated, the structures appear to be compatible with N deprotonation as well or a mixture of isomers. In fact, it may be that neither structure is accurate enough for a firm conclusion to be drawn. In this section, we resort to ab initio calculations to establish the structure of the [Gly−H+Zn]$^+$ complexes in aqueous solution.

In previous work, we have shown that isomers **1** and **2** have very similar energies with **2** marginally more stable than **1** by 5 kJ/mol. Although N-deprotonated glycine is more than 200 kJ/mol less stable than the O-deprotonated isomer [14], an amidate is a much stronger binder to Zn^{2+} than is a carboxylate [15], leading to a negligible energy difference between the corresponding isomers of [Gly−H+Zn]$^+$. When one methanol molecule is attached to zinc, [**1**, (CH_3OH)] was found to be more stable than [**2**, (CH_3OH)] by 4 kJ/mol. This difference is small enough that both isomers may be postulated to exist in solution. Indeed, solvation of either isomer could lead to very similar solute–solvent interactions, thus to very similar energies in the condensed phase. In this section we show that this is very unlikely to be the case.

Although the main precursors of [Gly−H+Zn]$^+$ (in a 50:50 vol water/methanol solution) involve solvation with methanol molecules, we considered hydrated complexes in what follows. Results with only one molecule of either solvent vindicate this choice, which is dictated by the tractability of computation on larger complexes. We have considered hydration of **1** and **2** with up to four water molecules. The first solvation shell of zinc is known to be fluctuent, with an average number of ligands lying between four and six. Here deprotonated Gly provides chelation to two sites, nitrogen and one of the oxygens, therefore a minimum of two solvent molecules is required. By considering up to four molecules, we try to saturate the zinc environment for a better relevance of the model. An approximate solvent model, the polarizable continuum model, was also used to obtain a qualitative idea of the effect that solvation may have on the tetrahydrated isomers.

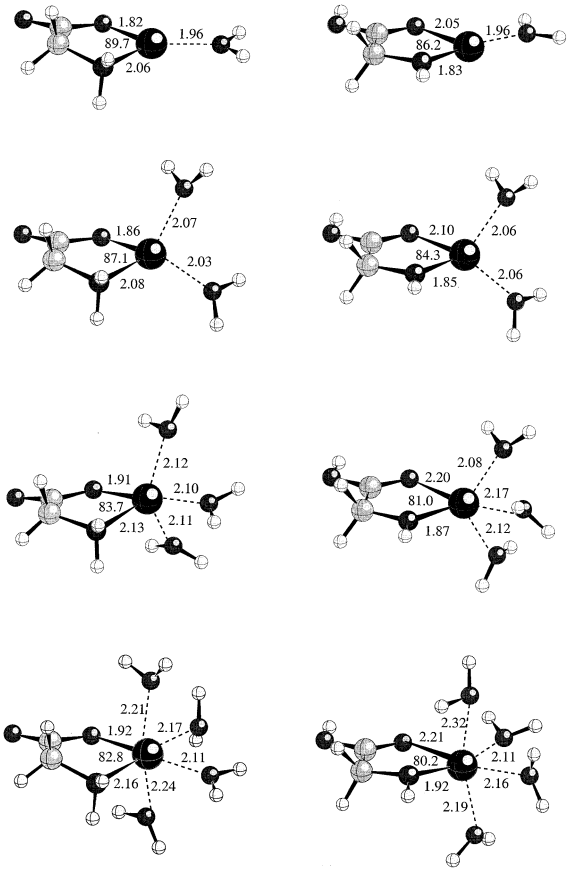

Fig. 2. Optimized geometries of hydrated complexes [**1**, $(H_2O)_n$] and [**2**, $(H_2O)_n$] with n−1,4 at the HF/basis1 level.

The optimized structures obtained at the HF/basis1 level for [**1**, $(H_2O)_n$] and [**2**, $(H_2O)_n$] (n=1–4) are shown in Figure 2. Their relative energies, and the successive water binding energies, are gathered in Table 1. Binding energies decrease significantly with increasing solvation as expected. This decrease is sharp since the first solvent molecule is bound by ∼180 kJ/mol (depending upon the isomer consid-

Table 1
Successive hydratation energies of isomers **1** and **2**: $D_e = E(H_2O) + E(\mathbf{X},H_2O)_{n-1} - E(\mathbf{X}, H_2O)_n$ ($\mathbf{X = 1,2}$); $D_0 = D_e + \Delta(ZPVE)$; $D_{298} = D_e + \Delta\ (E_{therm})$; $\Delta G_{298} = D_{298} - \Delta(T\Delta S)$ with $T = 298$ K. Except for the charges (in atomic units), all values are given in kJ/mol.

Species	ΔE_{298}[a]	Zn charge[b]	D_e	D_0	D_{298}	$-T\Delta S$	ΔG_{298}
1	0	1.664	···				
2	−5	1.648	···				
[**1,**H_2O]	0	1.657	193	183	183	−40	143
[**2,**H_2O]	1	1.618	187	176	177	−43	144
[**1,**(H_2O)–H_2O]	0	1.677	113	103	104	−40	64
[**2,**(H_2O)–H_2O]	19	1.655	95	86	86	−38	48
[**1,**$(H_2O)_2$–H_2O]	0	1.691	83	74	74	−42	32
[**2,**$(H_2O)_2$–H_2O]	27	1.661	75	65	66	−43	34
[**1,**$(H_2O)_3$–H_2O]	0	···	68	59	59	−45	14
[**2,**$(H_2O)_3$–H_2O]	40	···	57	46	47	−45	2

[a] Energy differences between [[**1,** $(H_2O)_n$] and [**2,** $(H_2O)_n$] with $n = 0$ to 4 at 298 K.
[b] Charges on Zn calculated with the NBO formalism.

ered), whereas the fourth binding energy is only worth ∼50−60 kJ/mol.

It can be seen that as the number of solvent molecules *n* increases, so does the energy difference between [**1**, $(H_2O)_n$] and [**2**, $(H_2O)_n$]. This is understandable because the charge on zinc, calculated with the NBO formalism, is always slightly larger in the complexes involving **1** than **2**. This is due to the stronger electron donation ability of deprotonated nitrogen than deprotonated oxygen. The difference in each successive binding energy between [**1**, $(H_2O)_n$] and [**2**, $(H_2O)_n$] is moderate, 11 kJ/mol in average. However the total energy difference between [**1**, $(H_2O)_4$] and [**2**, $(H_2O)_4$] amounts to 40 kJ/mol, a value which precludes any significant presence of [**2**, $(H_2O)_4$] if solvated **1** and **2** are in thermal equilibrium. Solvent modeling with the polarizable continuum model was also considered. Geometry optimization of tetrahydrated ions embedded in the continuum was carried out at the HF/basis1 level. **1** was found to be more stable than **2** by 138 kJ/mol. Although this value only has a qualitative significance [16], it makes it clear that only **1** is present in significant abundance in water solution.

3.2. Isomerization of [1, CH_3OH] into [2, CH_3OH]

The question thus remains, if **2** is the only possible precursor for loss of CO, how is it formed at all? Given the above results, it is unlikely that it is formed early in the electrospray process since the energy difference between solvated **2** and **1** is relatively large. Our previous computations of the direct interconversion between fully desolvated **1** and **2** indicated that it is prohibitively high, with an energy barrier of 215 kJ/mol relative to **1** [9]. Therefore we are led to focus on the final stages of desolvation, when one or just a few solvent molecules remain attached to zinc. The chemical change required for transforming **1** into **2** is a proton transfer from nitrogen to one of the carboxylate oxygens. However the initial position of both amino protons in **1**, at the exterior on the five-membered ring formed by Gly and zinc (see Fig. 1), makes direct proton transfer a highly distortive and therefore costly process. If, instead, proton transfer is directed to another zinc ligand, the necessary structural distortion will be much reduced, and so should be the energy required, too. This can be devised in the $[CH_3OH+Gly-H+Zn]^+$ precursor. The various isomers and transition states involved are gathered in Figs. 3 and 4, and their energies are given in Table 2.

Recall that there are several low energy isomers for the precursor ion, including [**1**, CH_3OH], [**2**, CH_3OH], and $[GlyZnOCH_3]^+$ in which it is the methanol ligand which is deprotonated. The most stable isomer of $[GlyZnOCH_3]^+$ is $[GlyA,CH_3OZn]^+$ as shown on Fig. 3. It is slightly more stable than [**1**, CH_3OH] and

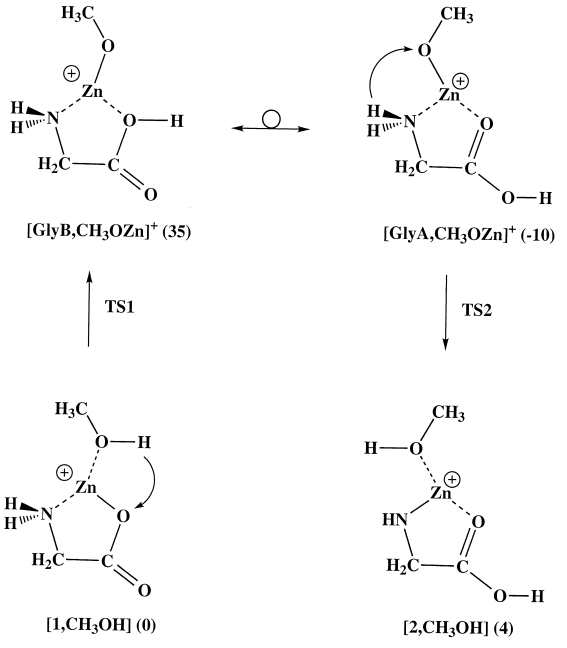

Fig. 3. Rearrangement of [**1**, CH_3OH] into [**2**, CH_3OH] by means of isomeric forms of [Gly, $CH_3OZn]^+$.

[**2**, CH_3OH], by 10 and 14 kJ/mol, respectively. From [**1**, CH_3OH], proton transfer from methanol to the carboxylate by way of **TS1** leads to a new isomer $[GlyB,CH_3OZn]^+$, in which it is the hydroxyl oxygen of Gly which is bound to Zn. Rotation of the methanolate ligand around the Zn-O bond and rotation of the carboxylic function around the C-C bond lead to $[GlyA,CH_3OZn]^+$. A second proton transfer is then possible from the amino group of Gly to the methanolate ligand, yielding [**2**, CH_3OH] by means of **TS2**. The activation barriers for these two proton transfers are 105 and 108 kJ/mol, respectively. Since evaporation of methanol from [**1**, CH_3OH] requires 217 kJ/mol, rearrangment to [**2**, CH_3OH] is strongly favoured.

*3.3. Desolvation versus rearrangement in [**1**, CH_3OH]*

We are now able to put together the various processes described above and in the previous articles [8,9] to obtain a complete picture of the formation of

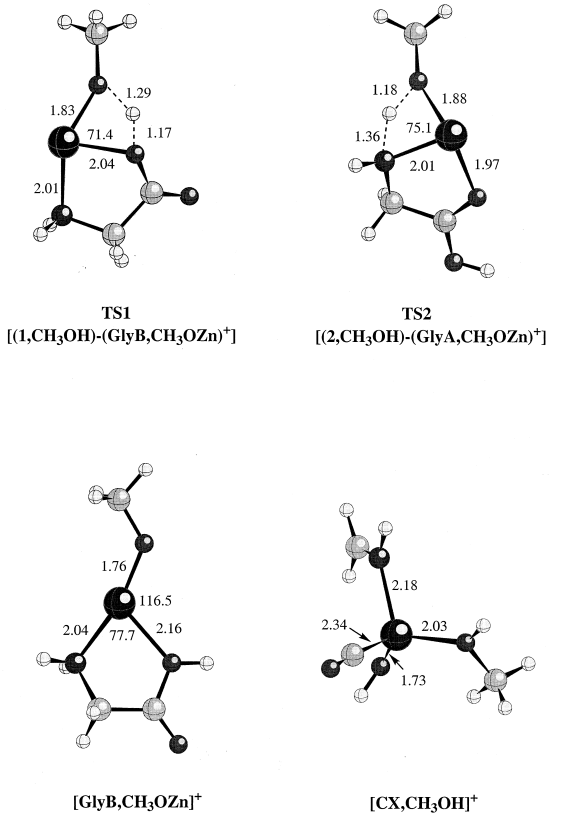

Fig. 4. Ball and stick geometries of **TS1**, **TS2**, [GlyB, $CH_3OZn]^+$ and [**CX**, CH_3OH].

$[Gly-H+Zn]^+$ and of its fragmentations when collisionally activated. This is gathered on Fig. 5. We start with the single isomer of $[CH_3OH+Gly-H+Zn]^+$, [**1**, CH_3OH], which arises from desolvation of the unique structure present in solution. Direct detachment of methanol from [**1**, CH_3OH] is more demanding (217 kJ/mol) than isomerization via either of two pathways. The first, detailed previously, involves proton transport from the amino to the carboxylate groups of glycine by means of the methanol relay, yielding [**2**, CH_3OH]. The rate-determining step for this isomerization has an activation energy of 108 kJ/mol (direct desolvation of Gly from the methanol–deprotonated isomer which is formed along the way can be excluded as it requires 376 kJ/mol). The [**2**, CH_3OH] ion may rearrange into [**CX**, CH_3OH], where **CX** stands for $[ZnOH(CO)(CH_2NH)]^+$. The

Table 2
Total (in hartrees) and relative (in kJ/mol) energies of the [(Gly–H+Zn) $(CH_3OH)^+$ system calculated at MP2(FC)/basis2//HF/basis1 level of theory

Species	MP2/basis2// HF/basis1	ΔE	ZPVE	ΔE + ZPVE	E_{therm}	ΔE + E_{therm}	$T\Delta S$	ΔG
[**1**,MeOH]	−2177.042 646	0	353	0	380	0	131	0
[**2**,MeOH]	−2177.039 150	9	349	5	376	4	128	7
[**3**,MeOH]	−2177.006 566	95	336	78	364	79	130	80
[**4**,MeOH]	−2177.018 393	64	335	46	365	31	133	29
$[GlyA,CH_3OZn]^+$	−2177.046 361	−10	353	−10	380	−10	131	−10
$[GlyB,CH_3OZn]^+$	−2177.029 162	35	353	35	380	35	132	34
[**CX**,MeOH]	−2177.031 710	29	329	5	366	15	151	−5
$[Gly,CH_2O,ZnH]^+$	−2177.033 845	23	336	6	365	8	131	8
$[Gly,ZnH]^+ + CH_2O$	−2176.998 491	116	329	92	356	92	172	51
Gly + $[CH_3OZn]^+$	−2176.897 955	380	344	371	368	368	176	323
1+MeOH	−2176.956 720	226	346	219	371	217	172	179
2+MeOH	−2176.956 828	225	340	212	366	211	173	169
3+MeOH	−2176.968 266	195	331	173	358	173	176	128
4+MeOH	−2176.979 268	166	330	143	357	143	173	101
CX+MeOH	−2176.984 371	153	323	123	355	128	192	67
TS1=TS([**1**,MeOH]–$[GlyB,CH_3OZn]^+$)	−2176.997 722	118	342	107	367	105	124	112
TS2=TS([**2**,MeOH]–$[GlyA,CH_3OZn]^+$)	−2176.994 928	125	339	111	366	108	123	116
TS([**1**,MeOH]–[**3**,MeOH])	−2176.970 268	190	333	170	361	171	129	173
TS([**3**,MeOH]–[**4**,MeOH])	−2176.971 623	186	331	164	360	166	132	165
TS([**2**,MeOH]–[**CX**,MeOH])	−2176.993 278	130	349	124	347	124	130	125
TS($[GlyA,CH_3OZn]^+$–$[Gly,CH_2O,ZnH]^+$)	−2176.988 330	143	338	128	363	126	124	133

activation barrier for this rearrangement is 124 kJ/mol (relative to [**1**, CH_3OH]). Thus it is less energy demanding than the desolvation of [**2**, CH_3OH] (211 kJ/mol). Desolvation of **CX** can then occur; it is less demanding than that of **1** or **2** because it occurs from a four-ligand complex. It is a general feature in such complexes that detachment of the last solvent molecule will only occur when there is a sufficient number of ligands on the metal ion. From **CX**, direct loss of CO is easy [9]. It is one of the observed fragmentations in the CID spectrum of $[Gly-H+Zn]^+$.

From [**1**, CH_3OH], a competitive pathway involves

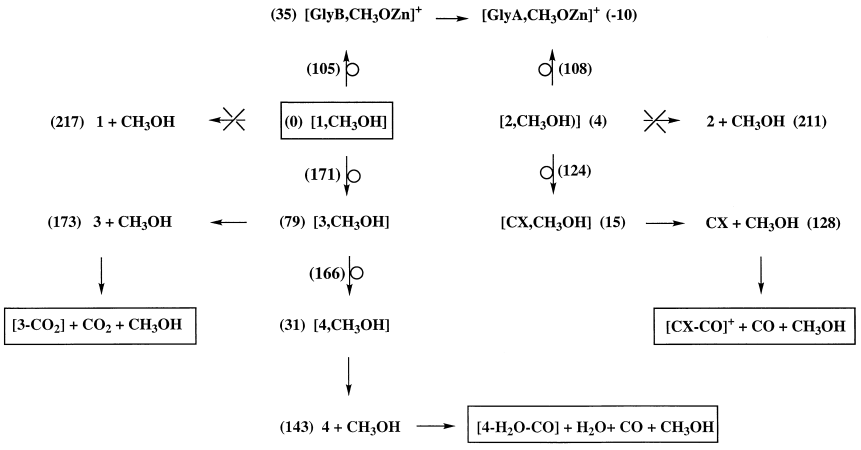

Fig. 5. Complete picture of isomerizations and fragmentations of $[(Gly-H+Zn)\ (CH_3OH)]^+$.

β-H migration from the CH_2 group of glycine to the metal, yielding [**3**, CH_3OH] with an activation barrier of 171 kJ/mol. This solvated unconventional isomer [**3**, CH_3OH] may either lose methanol to give **3** or rearrange into [**4**, CH_3OH] by hydrogen transfer from NH_2 to carboxylate. Desolvation and rearrangement require 173 and 166 kJ/mol, respectively. The desolvated structure **3** was demonstrated previously [9] to be the precursor for the loss of CO_2 whereas **4** is the one for the successive losses of H_2O and CO. **4** is formed either by desolvation of [**4**, CH_3OH] or by rearrangement of **3**. The easier process is the desolvation of [**4**, CH_3OH]. Then, the successive losses of H_2O and CO occur after rearrangement of **4** into a four-ligand complex in which HZn^+ interacts with H_2O, CO, and HCN [9].

The relative energies for this array of processes are such that the completely desolvated isomer **1** is never formed in the gas phase.

4. Conclusions

We have shown, in this and the previous articles in this series, that electrospray of a $ZnCl_2$/glycine mixture in H_2O/CH_3OH solution leads to the formation of gaseous $[Gly-H+Zn]^+$. The low energy collisional activation of this ion leads to three primary fragmentations, corresponding to losses of CO, CO_2, and H_2O+CO. Each of these fragmentations arises from a specific isomer of $[Gly-H+Zn]^+$, none of which corresponds to the liquid phase precursor. In the very final stages of desolvation, structural rearrangements occur yielding the three isomers eventually observed. One rearrangement, detailed in the present work, involves proton transfer between both end groups of glycine, using the last solvent molecule as a proton relay. It leads to the precursor of CO loss. The second rearrangement occurs by means of β-H migration to the metal, leading to unusual hydride-containing isomers. One is the precursor for loss of CO_2, the second leads to loss of H_2O+CO. Both mechanisms require activation barriers that are significantly smaller than the last desolvation energy.

This work shows that the formation of gaseous zinc complexes by electrospray may lead to unusual structures, resulting from isomerizations prior to evaporation of the last solvent molecule. This runs contrary to the common wisdom that gaseous electrosprayed ions are a direct image of solvated ions in solution. In fact, solvent evaporation requires significant amounts of energy, much like collisional activation in the "low energy" range (typically several electron volts). The last steps of solvent evaporation are expected to be the most demanding energetically, since such molecules are within the first solvation shell of the ion. In most cases of metal ions for which binding energetics are known, the last ligand is the most strongly bound. Therefore it is likely that it is in the very final stages of desolvation that isomerization will occur if any.

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