Gas Phase Investigation of [(Cu$^{2+}$, Ni$^{2+}$---Gly-Gly-His) – 3H$^+$]$^{-1}$ Complex by Electrospray Ionization MS/MS and MS/MS/MS

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Received January 30, 2007

Key Words : Gly-Gly-His, Metal$^{2+}$ ion, Metal-peptide complex, Mass spectrometry (MS), MS/MS

Mass spectrometry (MS) is a very useful means by which to study the interactions of metal cation-biomolecule complexes in the gas phase. The analysis of the fragmentation patterns of metal cationized peptides produced under electrospray ionization (ESI)-MS can provide complementary information for peptide sequencing when the fragmentation pattern of the protonated peptide is insufficient. The specific interactions in metal ion-peptide systems have been studied to develop practical sensors for the detection and quantification of metal ions.

Complexes of transition metal cations and peptides (transition metal$^{2+}$-- peptide)$^{2+}$ have been studied by many research groups. However, investigations regarding the [(Metal$^{2+}$-- peptide) – 3H$^+$]$^{-1}$ anion complex have not been conducted systematically using MS. The copper and nickel binding peptide Gly-Gly-His has been investigated in aqueous solution because the peptide Gly-Gly-His mimics the form of the specific Cu$^{2+}$, Ni$^{2+}$-transport active site of human serum albumin.

Theoretical studies concerning metal-oligopeptide structure and metal-ligand coordination geometry have also been performed through molecular dynamics simulations and $ab$ $initio$ calculations. Structures, molecular orbital and stabilization energies of metal-oligopeptides are reported by the research groups.

In this study, our attention was focused on the interaction between the oligopeptide of three amino acid residues Gly-Gly-His and metal ions (Cu$^{2+}$, Ni$^{2+}$) in the gas phase. The interaction between the Gly-Gly-His and metal ions was studied by ESI-MS in negative mode. The fragmentation pattern of the [(Cu$^{2+}$, Ni$^{2+}$---Gly-Gly-His) – 3H$^+$]$^{-1}$ anion complex was analyzed by MS/MS and MS/MS/MS spectra.

Experimental Section

The gas phase [(Metal$^{2+}$---Gly-Gly-His) – 3H$^+$]$^{-1}$ anion complex was produced by an electrospray ionization source. The experimental MS, MS/MS and MS/MS/MS data for fragmentation pattern analysis were obtained using a Thermo Finnigan LTQ mass spectrometer (Thermo Electron Corp., San Jose, CA, USA). This mass spectrometer is a linear ion trap mass spectrometer equipped with an atmospheric pressure-ionization source.

LTQ conditions. All spectra were acquired in negative ion mode over a range of m/z 100-400 by averaging 40 scans. The heated capillary temperature was set at 200 °C to facilitate efficient complex formation. The electrospray needle voltage was set at 3.3 kV. Nitrogen was used as the sheath gas (flow 20 units) and auxiliary gas (flow 5 units) in the electrospray ionization region. The samples were introduced into the electrospray interface by a direct infusion method using a microsyringe pump (SEGA, Australia) at a flow rate of 10 μL/min. The MS/MS spectra were acquired with experimental conditions of an isolation width of 1 mass unit, an activation time of 30 msec and q$^*$ = 0.25. In MS/MS mode, the parent ion molecules were manually selected one by one, and each was subjected to collision-induced dissociation (CID).

Reagents. Gly-Gly-His (99%, Sigma-Aldrich Korea), Cupric chloride dihydrate (99%, Sigma-Aldrich Korea), Nickel(II) nitrate hexahydrate (97%, Junsei chemical Co., Tokyo, Japan), Zinc nitrate hexahydrate (98%, Sigma-Aldrich Korea), Calcium chloride dihydrate (98%, Dae Jung chemical, Korea), and H$_2$O (HPLC grade, Merck) were used in experiments. Gly-Gly-His was dissolved in water to prepare a 2.4 M solution. The four metal solutions were prepared in aqueous solution are shown in Figure 1. The [(Cu$^{2+}$---Gly-Gly-His) – 3H$^+$]$^{-1}$ complex is seen to possess a planar structure involving the coordination of a terminal amino nitrogen, two deprotonated amide nitrogens, and the imidazole-N3 atom. The [(Cu$^{2+}$---Gly-Gly-His) – 3H$^+$]$^{-1}$ plane complex between Cu$^{2+}$ and four central nitrogen atoms (4 N) is known as the most stable structure in the four-coordination complex geometries.

Results and Discussion

The structural features of the [(Cu$^{2+}$---Gly-Gly-His) – 3H$^+$]$^{-1}$ complex in aqueous solution are shown in Figure 1. The [(Cu$^{2+}$---Gly-Gly-His) – 3H$^+$]$^{-1}$ complex was observed at m/z 329, m/z 324, m/z 330, m/z 306 and the [(Gly-Gly-His – H$^+$)]$^{-1}$ peptide ion was observed at m/z 268 (Fig. 2). The most meaningful observation gleaned from the MS spectra is that the formation efficiency of [(Cu$^{2+}$, Ni$^{2+}$---Gly-
Gly-His – 3H+\]^−_1 complex is much better than that of [Zn2+, Ca2+---(Gly-Gly-His – 3H+\)]^−_1 complex. The more than adequate formation efficiency of the [Cu2+, Ni2+---(Gly-Gly-His – 3H+\)]^−_1 complex was explained by the stabilization energy of the four-coordination planar structures in the [Cu2+, Ni2+---(Gly-Gly-His – 3H+\)]^−_1 complex. 15,19 The reason of bad formation efficiency of the [Zn2+---(Gly-Gly-His – 3H+\)]^−_1 complex is not clear in this step. The ratios of \[(Metal2+---Gly-Gly-His) – 3H+\]^−_1 peak area to \[(Gly-Gly-His – H+\)]^−_1 peak area + \[(Metal2+---Gly-Gly-His) – 3H+\]^−_1 peak area} are reported in Table 1. The metal isotope peak effects are also included in the area ratios. The adequate formation efficiency of the [Cu2+, Ni2+---(Gly-Gly-His – 3H+\)]^−_1 complex could explain why the specific Cu2+, Ni2+-transport active site of human serum albumin is similar to Gly-Gly-His peptide.12,13

The MS/MS spectra of [\((Metal2+---Gly-Gly-His) – 3H+\)]^−_1 complexes: (a) [\((Cu2+---Gly-Gly-His) – 3H+\)]^−_1 complex and (b) [\((Ni2+---Gly-Gly-His) – 3H+\)]^−_1 complex.

Table 1. The ratios of \[(Metal2+---Gly-Gly-His) – 3H+\]^−_1 peak area to \[(Gly-Gly-His – H+\)]^−_1 peak area + \[(Metal2+---Gly-Gly-His) – 3H+\]^−_1 peak area\] in Figure 2

<table>
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<tr>
<th>Metal</th>
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<tr>
<td>Cu2+</td>
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</tr>
<tr>
<td>Ni2+</td>
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<tr>
<td>Zn2+</td>
<td>0.093</td>
</tr>
<tr>
<td>Ca2+</td>
<td>0.091</td>
</tr>
</tbody>
</table>

Figure 3. MS/MS spectra of [\((Metal2+---Gly-Gly-His) – 3H+\)]^−_1 complexes: (a) [\((Cu2+---Gly-Gly-His) – 3H+\)]^−_1 complex and (b) [\((Ni2+---Gly-Gly-His) – 3H+\)]^−_1 complex.

Gly-His – 3H+\]^−_1 complex is much better than that of [Zn2+, Ca2+---(Gly-Gly-His – 3H+\)]^−_1 complex. The more than adequate formation efficiency of the [Cu2+, Ni2+---(Gly-Gly-His – 3H+\)]^−_1 complex was explained by the stabilization energy of the four-coordination planar structures in the [Cu2+, Ni2+---(Gly-Gly-His – 3H+\)]^−_1 complex.\textsuperscript{15,19} The reason of bad formation efficiency of the [Zn2+---(Gly-Gly-His – 3H+\)]^−_1 complex is not clear in this step. The ratios of [\((Metal2+---Gly-Gly-His) – 3H+\)]^−_1 peak area to \[(Gly-Gly-His – H+\)]^−_1 peak area + \[(Metal2+---Gly-Gly-His) – 3H+\]^−_1 peak area\] are reported in Table 1. The metal isotope peak effects are also included in the area ratios. The adequate formation efficiency of the [Cu2+, Ni2+---(Gly-Gly-His – 3H+\)]^−_1 complex could explain why the specific Cu2+, Ni2+-transport active site of human serum albumin is similar to Gly-Gly-His peptide.\textsuperscript{12,13}

The MS/MS spectra of [\((Metal2+---Gly-Gly-His) – 3H+\)]^−_1 complex are shown in Figure 3. The fragment ions at m/z 285 in Figure 3a and at m/z 280 in Figure 3b are thought to be a result of the common loss of a CO2 moiety from the [\((Cu2+, Ni2+---Gly-Gly-His) – 3H+\)]^−_1 complex at the low collision activation energy. Yang et al. reported that the fragment ion of a 44u loss corresponds to a decarboxylation from the histidine residue.\textsuperscript{7} In their previous works, the CO2-loss fragment of m/z 285 was reported as the one of several fragments of the [\((Cu2+---Gly-Gly-His) – 3H+\)]^−_1 parent ion because of the uncontrolled collision activation energy in the anion formation MS spectrum. It is worth noting that the C-CO2 bond of the [\((Cu2+, Ni2+---Gly-Gly-His) – 3H+\)]^−_1 complex was found to be the weakest bond of the [\((Cu2+, Ni2+---Gly-Gly-His) – 3H+\)]^−_1 complex in our low energy CID-MS/MS spectra.

The MS/MS/MS spectra of the CO2-loss fragment that
originated from the \([\text{Cu}^{2+}, \text{Ni}^{2+}---\text{Gly-Gly-His}) – 3\text{H}^+ – \text{CO}_2\] complex are shown in Figure 4. It is assumed that the observed fragments of m/z 251, m/z 223, m/z 194 in Figure 4b) are the \(x_2, y_2\) and \(x_1\) ions of the \([\text{Ni}^{2+}---\text{Gly-Gly-His}) – 3\text{H}^+ – \text{CO}_2\] complex. However, the main fragment of the \([\text{Cu}^{2+}---\text{Gly-Gly-His}) – 3\text{H}^+ – \text{CO}_2\] complex in a) was observed at m/z 241. The fragment of m/z 241, the ion resulting from a 44u loss from the \([\text{Cu}^{2+}---\text{Gly-Gly-His}) – 3\text{H}^+ – \text{CO}_2\] complex, is not a fragment normally obtained in the peptide dissociation in a typical MS spectrum. The additional 44u-loss could be explained by a \(\text{C}_2\text{H}_4\text{NH}_2\), or \(\text{HCONH}\), or \(\text{HCOCH}_3\) loss from the \([\text{Cu}^{2+}---\text{Gly-Gly-His}) – 3\text{H}^+ – \text{CO}_2\] complex. It is difficult to address the mechanism for the formation of these ions because of the lack of information in the collision-induced dissociation spectra. Further experimentation is needed for a better understanding of the fragmentation patterns in the \([\text{Cu}^{2+}---\text{Gly-Gly-His}) – 3\text{H}^+ – \text{CO}_2\] MS/MS/MS spectrum.

In summary, the adequate formation efficiency of the \([\text{Cu}^{2+}, \text{Ni}^{2+}---(\text{Gly-Gly-His} – 3\text{H}^+)\] complex in the gas phase MS spectra reflects what is also observed in the solution phase absorption spectra. The C-CO\(_2\) bond is found to be the weakest bond of the \([\text{Cu}^{2+}, \text{Ni}^{2+}---\text{Gly-Gly-His}) – 3\text{H}^+]\) complex in our low energy CID-MS/MS spectra. The structure of the \([\text{Cu}^{2+}, \text{Ni}^{2+}---\text{Gly-Gly-His}) – 3\text{H}^+]\) complex in the gas phase was assumed to maintain the planar structure it held in the solution phase on the basis of the analysis of the MS and MS/MS spectra.

Acknowledgements. This paper was supported by Research Fund, Kumoh National Institute of Technology.

References