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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Mass spectrometry (MS) is a very useful means by which to study the interactions of metal cation-biomolecule complexes in the gas phase.[1,2] 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 of the protonated peptide is insufficient.[3,4] The specific interactions in metal ion-peptide systems have been studied to develop practical sensors for the detection and quantification of metal ions.[5-7]

Complexes of transition metal cations and peptides (transition $metal^{2+}$--- peptide)$^{2+}$ have been studied by many research groups.[8,9] However, investigations regarding the $[(Metal^{2+}$--- peptide) – $3H^+]^{-1}$ anion complex have not been conducted systematically using MS.[7,10,11] 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.[12,13]

Theoretical studies concerning metal-oligopeptide structure and metal-ligand coordination geometry have also been performed through molecular dynamics simulations and *ab initio* calculations.[14-17] 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 elctrospray interface by a direct infusion method using a microsyringe pump (SEG, Australia) at a flow rate of 10 mL/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 $q_z = 0.25$. 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_2O (HPLC grade, Merck) were used in experiments. Gly-Gly-His was dissolved in water to prepare a 2.4×10^{-4} M solution. The four metal solutions were prepared in water at a final concentration of 2.4×10^{-4} M. These two solutions were mixed together prior to obtaining the mass spectra.

Results and Discussion

The structural features of the $[(Cu^{2+}$---Gly-Gly-His) – $3H^-]^{-1}$ complex in aqueous solution are shown in Figure 1.[18,19] 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) – $3H^-]^{-1}$ planar complex between Cu^{2+} and four central nitrogen atoms (4 N) is known as the most stable structure in the four-coordination complex geometries.

Negative mode MS spectra of four metal ion complexes in aqueous solution are shown in Figure 2. The $[(^{63}Cu^{2+}, ^{58}Ni^{2+}, ^{64}Zn^{2+}, Ca^{2+}$---Gly-Gly-His) – $3H^-]^{-1}$ complexes were observed at m/z 329, m/z 324, m/z 330, m/z 306 and the $[(Gly\text{-}Gly\text{-}His - H^-)]^{-1}$ 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-

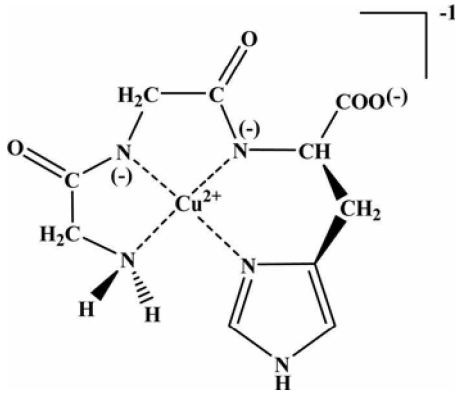

Figure 1. Structure of $[(Cu^{2-}\text{---Gly-Gly-His}) - 3H^+]^{-1}$ complex in aqueous solution.

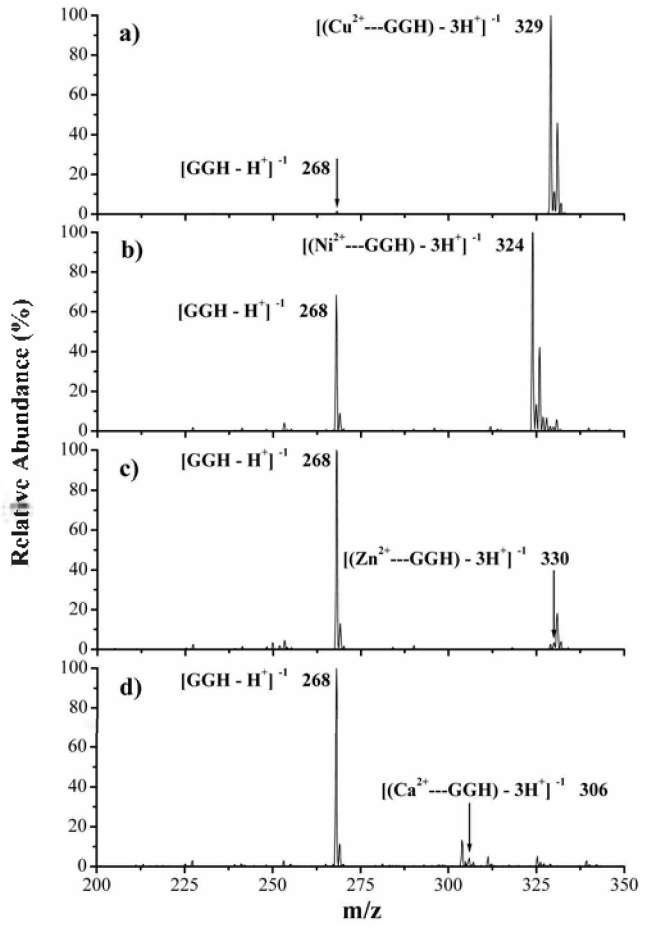

Figure 2. MS spectra in negative mode: (a) Cu^{2-} ion + Gly-Gly-His, (b) Ni^{2+} ion + Gly-Gly-His, (c) Zn^{2-} ion + Gly-Gly-His and (d) Ca^{2+} ion + Gly-Gly-His.

Gly-His – $3H^-)]^{-1}$ complex is much better than that of $[Zn^{2+}$, Ca^{2-}---(Gly-Gly-His – $3H^+)]^{-1}$ complex. The more than adequate formation efficiency of the $[Cu^{2-}$, Ni^{2-}---(Gly-Gly-His – $3H^+)]^{-1}$ complex was explained by the stabilization energy of the four-coordination planar structures in the $[Cu^{2-}$, Ni^{2-}---(Gly-Gly-His – $3H^-)]^{-1}$ complex.[15,19] The reason of bad formation efficiency of the $[Zn^{2-}$---(Gly-Gly-His – $3H^+)]^{-1}$ complex is not clear in this step. The ratios of

Table 1. The ratios of $[(Metal^{2-}\text{---Gly-Gly-His}) - 3H^+]^{-1}$ peak area to $\{[(Gly\text{-}Gly\text{-}His - H^-)]^{-1}$ peak area + $[(Metal^{2-}\text{---Gly-Gly-His}) - 3H^-]^{-1}$ peak area$\}$ in Figure 2

	Peak Area $[(Metal^{2+}\text{---Gly-Gly-His})\text{-}3H^+]^{-1}$ / Peak Area $\{[(Gly\text{-}Gly\text{-}His - H)^-]^{-1}$ + $[(Metal^{2+}\text{---Gly-Gly-His})\text{-}3H^+]^{-1}\}$
Cu^{2-}	0.992
Ni^{2-}	0.689
Zn^{2-}	0.093
Ca^{2-}	0.091

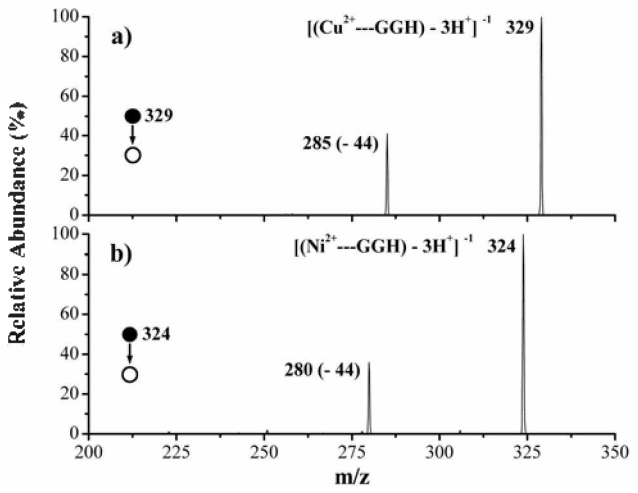

Figure 3. MS/MS spectra of $[(Cu^{2-}, Ni^{2-}\text{---Gly-Gly-His}) - 3H^-]^{-1}$ complexes: (a) $[(Cu^{2-}\text{---Gly-Gly-His}) - 3H^-]^{-1}$ complex and (b) $[(Ni^{2-}\text{---Gly-Gly-His}) - 3H^-]^{-1}$ complex.

$[(Metal^{2+}\text{---Gly-Gly-His}) - 3H^+]^{-1}$ peak area to $\{[(Gly\text{-}Gly\text{-}His - H^-)]^{-1}$ peak area + $[(Metal^{2+}\text{---Gly-Gly-His}) - 3H^+]^{-1}$ 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 $[Cu^{2+}$, Ni^{2-}---(Gly-Gly-His – $3H^-)]^{-1}$ complex could explain why the specific Cu^{2+}, Ni^{2-}-transport active site of human serum albumin is similar to Gly-Gly-His peptide.[12,13]

The MS/MS spectra of $[(Metal^{2+}\text{---Gly-Gly-His}) - 3H^+]^{-1}$ 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 CO_2 moiety from the $[(Cu^{2-}$, $Ni^{2+}\text{---Gly-Gly-His}) - 3H^-]^{-1}$ 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.[7] In their previous works, the CO_2-loss fragment of m/z 285 was reported as the one of several fragments of the $[(Cu^{2+}\text{---Gly-Gly-His}) - 3H^+]^{-1}$ parent ion because of the uncontrolled collision activation energy in the anion formation MS spectrum. It is worth noting that the C-CO_2 bond of the $[(Cu^{2-}$, $Ni^{2+}\text{---Gly-Gly-His}) - 3H^-]^{-1}$ complex was found to be the weakest bond of the $[(Cu^{2+}$, $Ni^{2-}\text{---Gly-Gly-His}) - 3H^+]^{-1}$ complex in our low energy CID-MS/MS spectra.

The MS/MS/MS spectra of the CO_2-loss fragment that

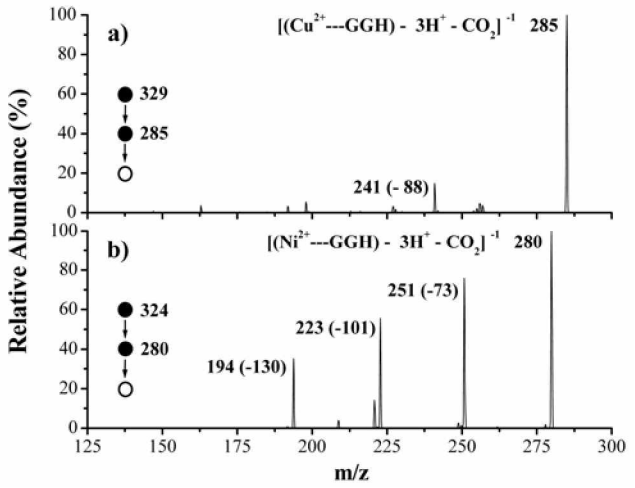

Figure 4. MS/MS/MS spectra of $[(Cu^{2+}, Ni^{2+}$---Gly-Gly-His) – $3H^+ - CO_2]^{-1}$ complexes: (a) $[(Cu^{2+}$---Gly-Gly-His) – $3H^+ - CO_2]^{-1}$ complex and (b) $[(Ni^{2+}$---Gly-Gly-His) – $3H^- - CO_2]^{-1}$ complex.

originated from the $[(Cu^{2+}, Ni^{2+}$---Gly-Gly-His) – $3H^+]^{-1}$ 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 $[(Ni^{2+}$---Gly-Gly-His) – $3H^+ - CO_2]^{-1}$ complex. However, the main fragment of the $[(Cu^{2+}$---Gly-Gly-His) – $3H^+ - CO_2]^{-1}$ complex in a) was observed at m/z 241. The fragment of m/z 241, the ion resulting from a 44u loss from the $[(Cu^{2+}$---Gly-Gly-His) – $3H^+ - CO_2]^{-1}$ 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 $C_2H_4NH_2$, or HCONH, or $HCOCH_3$ loss from the $[(Cu^{2+}$---Gly-Gly-His) – $3H^+ - CO_2]^{-1}$ 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 $[(Cu^{2+}$---Gly-Gly-His) – $3H^- - CO_2]^{-1}$ MS/MS/MS spectrum.

In summary, the adequate formation efficiency of the $[Cu^{2+}, Ni^{2+}$---(Gly-Gly-His – $3H^-)]^{-1}$ 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 $[(Cu^{2+}, Ni^{2+}$---Gly-Gly-His) – $3H^+]^{-1}$ complex in our low energy CID-MS/MS spectra. The structure of the $[(Cu^{2+}, Ni^{2+}$---Gly-Gly-His) – $3H^-]^{-1}$ 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.

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