Determination of a Binding Site of Cu, Ni, Mg, and Ca Metal Ions with Angiotensin II Peptide by Electrospray Tandem Mass Spectrometry

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Angiotensin II (Ang II) is the main active hormone in the renin-angiotensin blood pressure regulation system. Ang II is an octapeptide hormone ([Asp1-Ang2-Val3-Tyr4-Ile5-His6-Pro7-Phe8, DRVYIHPF]) that has been studied for several decades in the structure-dependent activity of the biological pathway of the Ang II receptor. Free Ang II molecular structures in solutions have been investigated with a variety of techniques. The results have been reported as β-turn, random coil, hair-pin, and other structures. The structures of Ang II complexed with a receptor have been reported as a hair-pin or an extended structure in NMR and crystallography studies. It is evident that several reports are not consistent with each other and that no clear agreement exists regarding the biologically active structure of Ang II during the biological process.

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The present paper focuses on the binding sites between the metal ions (Cu$^{2+}$, Ni$^{2+}$, Mg$^{2+}$, and Ca$^{2+}$) and the Ang II molecule to explain the biological effect in terms of a gas-phase structural difference. We present the results of a tandem mass spectrometry and multiple mass spectrometry study regarding the interaction of metal ions with the Ang II molecule, the C-terminus amidated Ang II molecule (DRVYIHPF-NH$_2$), and two of its segments: the N-terminus tetrapeptide Asp1-Ang2-Val3-Tyr4-NH$_2$ (DRVY-NH$_2$) and the N-terminus hexapeptide Asp1-Ang2-Val3-Tyr4-Ile5-His6-NH$_2$ (DRVYIHNH$_2$). Two N-terminus segments are used to confirm the [metal$^{II}$ + Ang II]$^{2+}$ complex formation efficiency and the metal binding site.

Experimental Section

The gas-phase [metal$^{II}$ + Ang II]$^{2+}$ cation complexes were produced by an electrospray ionization source. The experimental MS/MS and MS/MS/MS spectra for fragmentation pattern analysis were obtained using a Thermo Finngan 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. The samples were introduced into the electrospray interface by a direct infusion method using a microsyringe pump (SGE, Australia) at a flow rate of 5 μL min$^{-1}$. The MS/MS spectra were acquired with experimental conditions of an isolation width of 0.5 - 1 mass unit, an activation time of 30 msec and q$_{p}$ = 0.25. In MS/MS or MS/MS/MS mode, the parent ion molecules were manually selected one by one, and each was subjected to collision-induced dissociation (CID).

Reagents. Ang II human (96%, Fluka), DRVYIHPF-NH$_2$ (>90%, Peptron, Daejeon, Korea), DRVY-NH$_2$ (>90%, Peptron, Daejeon, Korea), DRVYIHNH$_2$ (>90%, Peptron, Daejeon, Korea), CuCl$_2$·2H$_2$O (99%, Sigma-Aldrich Korea), Ni(NO$_3$)$_2$·6H$_2$O (97%, Junsei chemical Co., Tokyo, Japan), CaCl$_2$·2H$_2$O (98%, Daejung Chemical Korea), Mg(NO$_3$)$_2$·6H$_2$O (98%, Junsei chemical Co., Tokyo, Japan), and H$_2$O (HPLC grade, Merek) were used in experiments. Ang II was dissolved in water to prepare a 2 × 10$^{-5}$ M solution. The three metal (Ni$^{2+}$, Mg$^{2+}$, and Ca$^{2+}$) solutions were prepared at a final concentration of 4 × 10$^{-5}$ M in water. These two solutions were mixed together prior to obtaining the mass spectra. Cu$^{2+}$ solution was prepared at a final concentration of 4 × 10$^{-4}$ M in water.

Results and Discussion

The two-dimensional S-shape (with two turns) structural feature of Ang II was reported in a NMR experiment. Each turn could be an adequate binding site for metal ions. [metal$^{II}$ + Ang II]$^{2+}$ complexes are dominantly observed in the positive-mode ESI-MS spectrum in Figure 1. In the formation of [metal$^{II}$ + Ang II]$^{2+}$ complex, the neutral structure of Ang II molecule is supposed to be a more favorable form compared to the deprotonated form (Ang II – H$^+$) of the Ang II molecule in an aqueous solution (The intensity of [metal$^{II}$ + (Ang II – H$^+$)]$^{2+}$ complex is negligible in Figure 1). The metal binding sites of the [metal$^{II}$ + Ang II] complex were analyzed as the nitrogen atom coordination of the peptide bond in potentiometric experiments. In contrast to the potentiometric experimental results, the metal binding sites of the [metal$^{II}$ + peptide]$^{2+}$ complex were proposed as the oxygen atom coordination of the

Notes

peptide bond of the neutral peptide molecule according to mass spectrometry and molecular dynamics simulation results.\textsuperscript{21,23}

In Figure 2, the $[b_6 + Cu, Ni - H]^2^+$ fragment and $[y_7 + Mg + H]^2^+$ fragment ions were observed as the typical fragment ions in the MS/MS spectra of the parent $[Cu^+, Ni^{2+} + Ang II]^{2+}$ and $[Mg^{2+} + Ang II]^{2+}$ ions, respectively.\textsuperscript{3} The $y_7$ and $b_6$ ions were the main fragments in the MS/MS spectra of the parent $[Ang II + H]^+$ ions due to the five-membered ring cleavage of the Asp1 residue\textsuperscript{8,21,22,23} and the His6-containing cleavage,\textsuperscript{24,25} respectively. The single observation of the $[b_6 + Cu, Ni - H]^2^+$ (except $[y_7 + Cu, Ni + H]^2^+$ fragment ion) in Figure 2a and 2b might indicate that the approximate metal ($Cu^+$ and $Ni^{2+}$) binding sites contain the Asp1 residue. Correspondingly, the dissociation process (for the observation of $[y_7 + Cu, Ni + H]^2^+$ fragment ion) originated from the Asp1 five-membered ring cleavage is not supposed to be activated in the MS/MS spectra of the parent $[Cu^+, Ni^{2+} + Ang II]^{2+}$ ions.

Two extra fragmentation channels, $[Cu^{II} + Ang II - CO_2]^{2+}$ and $[Ca^{II} + Mg^{II} + Ang II - H_2O]^{2+}$ ions, were also observed in the MS/MS spectra of the parent $[Cu^{II}, Ca^{II}, Mg^{II} + Ang II]^{2+}$ ion. In the MS/MS spectra of the parent $[Ca^{II} + Ang II]^{2+}$ ion (Figure 2d), no observation of the $y_7$- or $b_6$-related fragment ions suggest that the dissociation processes originated from the Asp1 five-membered ring cleavage and the His6-containing cleavage are not activated due to the Ang II binding sites of the Ca$^{2+}$ ion. The Ang II binding sites of the Ca$^{2+}$ ion were reported to be two carboxyl groups (Asp1 and Phe8) and two peptide carboxyl oxygens (Arg2 and His6) in the NMR experiment.\textsuperscript{26}

In the MS/MS spectra of the $[b_6 + Cu - H]^2^+$ and $[b_6 + Ni - H]^2^+$ ions (Figure 3a and 3b), the observation of the $[as + Cu - H]^2^+$ fragment and the $[b_6 + Ni - 2H]^+$ fragment ions suggests that the Asp1-Ang2-Val3-Tyr4 sequence of Ang II could be assigned as a main binding site of the Cu$^{2+}$ and Ni$^{2+}$ ions, respectively, in the $[Cu^{II}, Ni^{2+} + Ang II]^{2+}$ gas-phase complex. In the case of the $[y_7 + Mg + H]^2^+$ ion (Figure 3c), the observation of the $[y_6 + Mg]^2^+$ fragment and the $[y_6 + Mg]^2^+$ fragment ions suggests that the Ile5-His6-Pro7-Phex8 sequence of Ang II is a main binding site of the Mg$^{2+}$ ions in the $[Mg^{2+} + Ang II]^{2+}$ gas-phase complex. In Figure 3d, NH$_3$ loss (~17 amu) and $[Ca^{II} + Ang II - H_2O - 60]^2^+$ fragment ions are observed at the MS/MS/MS spectra of $[Ca^{II} + Ang II - H_2O]^{2+}$. A loss of 60 amu is regarded as a combinational (NH$_3$ + side alkyl group) loss like as $[NH_3 + C(NH_3)NH]$ or $[NH_3 + CH(CH_2)_{15}]$. Two main fragment ions (~17-60 amu) are also observed in the MS/MS/MS spectrum (Figure 3e) of $[Ca^{II} + Ang II - H_2O - NH_3]^2^+$ ion. So, it is difficult to analyze the Ca$^{2+}$ ion binding sites of
Ang II due to the dissociation channels related to H$_2$O and NH$_3$ loss instead of the peptide backbone dissociation channel in the MS/MS and MS/MS/MS spectra.

The MS/MS spectrum of [Cu$^{II}$ + DRVYIH-PF-NH$_2$]$^{2+}$ is observed to investigate the CO$_2$ dissociation process. Figure 4a spectrum is identical to that in Figure 2a except for the ~44 amu (CO$_2$ loss) fragment ion. C-terminus amiation blocking prominently decreases the ~44 amu fragmentation channel. Therefore, it is concluded that the fragmentation channel of CO$_2$ loss arises from the C-terminus carboxyl group instead of from the Asp1 side chain carboxyl group, which involves intramolecular hydrogen bonding with the Arg2 backbone peptide bond.

The analogous MS/MS fragmentation patterns of [Ni$^{II}$, Mg$^{2+}$, Ca$^{2+}$ + DRVYIH-PF-NH$_2$]$^{2+}$ (Figure 4b, 4c, and 4d) by comparing MS/MS spectra of [Ni$^{II}$, Mg$^{2+}$, Ca$^{2+}$ + Ang II]$^{2+}$ ions (Figure 2b, 2c, and 2d) suggest that the C-terminus amiation blocking does not make any influence in the binding site propensities of Ni$^{2+}$, Mg$^{2+}$, and Ca$^{2+}$ ions regarding the formation of [metal$^{II}$ + Ang II]$^{2+}$ complexes.

Positive-mode ESI-MS spectra of DRVY-NH$_2$ and DRVYIH-NH$_2$ complexed by Cu$^{2+}$ or Ni$^{2+}$ ions are shown in Figure 5a and 5b. The metal (Cu$^{2+}$ or Ni$^{2+}$) complex formation efficiency (which is observed as an [Cu$^{II}$, Ni$^{II}$ + DRVY-NH$_2$]$^{2+}$ ion intensity) of peptide segment DRVY-NH$_2$ is observed to be similar to that of peptide segment DRVYIH-NH$_2$. The MS/MS spectra (Figure 5c and 5d) of the parent [Cu$^{II}$, Ni$^{II}$ + DRVYIH-NH$_2$]$^{2+}$ ions show that the Cu$^{2+}$ and Ni$^{2+}$ binding sites of Ang II is identical to that of [Cu$^{II}$, Ni$^{II}$ + DRVYIH-PF-NH$_2$]$^{2+}$ (Figure 4a and 4b). Therefore, it is supposed that the N-terminus vicinity of Ang II is the better binding site in the competition between the N-terminus vicinity and the His9 vicinity of Ang II to anchor Cu$^{2+}$ or Ni$^{2+}$ metal ion because the metal ion (Cu$^{2+}$ or Ni$^{2+}$) are regarded to interact with peptide carboxyl oxygens instead of peptide nitrogens at neutral pH (where the peptide bonds are not deprotonated).

As a concluding remark, the structures of [Cu$^{II}$, Ni$^{II}$ + Ang II]$^{2+}$ complex were investigated by comparing those of [Ca$^{2+}$, Mg$^{2+}$ + Ang II]$^{2+}$ complex in the gas-phase CID mass spectra. The [b$_6$ + Cu, Ni - H]$^{+}$, [a$_3$ + Cu + H]$^{+}$ and [b$_4$ + Ni - 2H]$^{+}$...
activity would not be enhanced by the addition of Cu²⁺ or Ni²⁺ ions.

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References