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Formation of $[nM-nH+(n+1)Na]^+$ cluster ions from amino acid by electrospray ionization

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ABSTRACT

Twenty proteinic amino acids were ionized in the presence of sodium cation by electrospray ionization (ESI) method and the product ions were analyzed. Among them, asparagine, glutamine, serine, and threonine generated the $[nM-nH+(n+1)Na]^+$ cluster ions. The cluster ion intensity distributions showed a magic number at $n=3$ of six-coordinated sodium complex structures, $(M-H+Na)_3Na^+$. The neutral species of (M−H+Na) formed by proton-sodium cation exchange of asparagine, glutamine, serine, and threonine could have stable cyclic structures of 7-, 8-, 6-, and 6-membered rings, respectively. They also had two ligand sites of ketone and amine. We concluded that formation of the proton-sodium cation exchanged species plays an important role in the cluster ion formation.

1. Introduction

The interactions of alkali metal cations with organic molecules in the gas phase have attracted considerable attention. Since the introduction of soft ionization methods such as fast atom bombardment (FAB), electrospray ionization (ESI), and matrix-assisted laser desorption/ionization (MALDI) [1–3], metal complexation has been used as an analytical tool to enhance the characterization of complex bioactive molecules [4]. The fragmentation pathways of protonated and metal-cationized molecules are often different and provide complementary information [5].

The sodium cation is a ubiquitous contaminant of samples in electrospray mass spectrometry. For peptide samples, the presence of high concentrations of Na^+ typically results in envelopes of ions of $[M-nH+mNa]^{(m-n)+}$, where n is the number of protons abstracted from the peptide M, and m is the number of sodium ions incorporated [6,7]. There is no known general binding of alkali metal cations to peptides in solution. For the specific cases in which in vivo binding is known, the numbers of bound sodium or potassium cations are small [8,9]. By contrast, it is known that transition metal cations bind strongly to peptides in solution; anchoring a transition metal cation at the N-terminus of a peptide is believed to be the first step leading to subsequent deprotonation of an amide linkage and chelation of the metal ion. Similar deprotonation reactions in solution have not been reported for alkali metals despite experiments aimed at finding them [10]. The apparent simplicity of the electrospray mass spectrum has helped to mask the complexity of electrospray mass spectrometry, which is slowly being recognized. Some studies [8,11–13] reported dimeric protein ions that are isobaric with monomeric ions. Thomson [12] and Ke and coworkers [14] observed that the apparent continuous background in an electrospray mass spectrum recorded under mild lens conditions contains solvated multimetric ions of the analyte. Zhan and coworkers [15] reported re-solvation of bare electrospray-generated peptide ions in the lens region of the mass spectrometer that resulted from nucleation within the supersonic expansion jet. Analysis of amino acids using liquid chromatography/mass spectrometry (LC/MS) has been studied [16–23]. Some researchers [17,18] anaylzed underivatized amino acids for the diagnosis and therapy assessment. Protonated cluster ions of amino acids, $[nM+kH]^{k+}$ were reported [21,22] and Nanita and coworkers reported alkali metal-cationized serine clusters [23].

In this work, we tried to find the proton-sodium exchanged species of amino acid and the related cluster ions in electrospray mass spectrometry. Twenty proteinic amino acids were analyzed. In order to enhance the proton-sodium exchange reactions, sodium salt (NaOH) was added to the amino acid solution and the concentration varied. Unlike the peptides, the $[M-nH+mNa]^{(m-n)+}$ species were not observed. However, the $[nM-nH+(n+1)Na]^+$ cluster ions were observed.

2. Experimental

Glycine, phenylalanine, tyrosine, tryptophan, serine, and valine were purchased from Daejung Chemicals & Metals Co. (Korea). Arginine, aspartic acid, glutamic acid, and proline were purchased from Samchun Pure Chemical Co. (Korea). Alanine, asparagine, glu-

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tamine, histidine, leucine, and threonine were purchased from Junsei Chemaical Co. (Japan). Isoleucine and lysine were purchased from Acros Organics (USA), and cysteine was purchased from Merck Co. (Germany). Methionine was purchased from Yakuri Pure Chemicals Co. (Japan). The 20 underivatized amino acids were used as received without further purification. HPLC grade acetonitrile and deionized water were obtained from J.T. Baker & Co. (USA). Amino acid solutions of 5.0 mM were prepared by dissolving the 20 underivatized amino acids in deionized water. NaOH solutions of 0.25, 0.5, and 1.0 mM were also prepared by dissolving them in deionized water. The amino acid and NaOH solutions with the same volume were mixed.

An Agilent Technologies (CA, USA) 1100 Series LC/MSD system consisting of a vacuum degasser, a quaternary solvent pump, an autosampler with a column oven, and a MSD coupled with an analytical workstation was used. The liquid chromatograph with a binary pump was used and 10 μL of the sample solution was directly injected using an autosampler. The mass detection system was equipped with a standard ESI source. All mass spectra were recorded under identical analytical conditions. Parameters included: flow rate, 0.7 mL/min; sheath gas pressure 120 psi; heated capillary temperature 350 °C; spray voltage 3.5 kV; fragmentor voltages, 30 and 50 V. Experiments were performed three times and averaged.

3. Results and discussion

The amino acid-sodium cation solution was ionized by the ESI method. Twenty proteinic amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) were analyzed. The ESI mass spectra displayed the sodiated molecule, $[M+Na]^+$ as well as the protonated molecule, $[M+H]^+$. Among them, the mass spectra of asparagine, glutamine, serine, and threonine showed the cluster ion series of $[nM-nH+(n+1)Na]^+$ as shown in Fig. 1. The $[nM-nH+(n+1)Na]^+$ ions are the sodium complex of (M−H+Na) which is formed by proton-sodium cation exchange. Besides ESI, sodiated amino acids were also generated by MALDI [24] and FAB [25]. The $[nM-nH+(n+1)Na]^+$ ions of amino acids have not been reported, but the $[nM-nH+(n+1)Na]^+$ ions of peptides such as achatin-I [26] and phosphotyrosine peptide [27] were reported. Protonated cluster ions, $[nM+H]^+$ of amino acids generated by ESI were reported [21,22]. Concina and coworkers [22] also observed $[nM+Na]^+$, $[nM+H+Na]^{2+}$, and $[nM+H+2Na]^{3+}$ of serine. Cooks and coworkers reported sodiated serine clusters as well as protonated serine clusters using LC/ESI-MS and ion mobility-mass spectrometry [28,29]. Nanita and coworkers [23] studied formation of alkali metal-cationized serine clusters by sonic spray ionization (SSI) and reported $[Ser_n+Cat]^+$ of Li^+, Na^+, K^+, Rb^+, and Cs^+. We observed cluster ions of the proton-sodium cation exchanged molecules of amino acids, $[nM-nH+(n+1)Na]^+$. However, cluster ions of the proton-sodium cation exchanged molecules of amino acids have not been reported by other research groups. Proton-sodium cation exchanged species (M−H+Na) can have cyclic structures as shown in Scheme 1. It can be expected that cysteine also produces similar cluster ions as serine, because cysteine ($HO_2CCH(NH_2)CH_2SH$) has a very similar structure as serine ($HO_2CCH(NH_2)CH_2OH$). However, cysteine did not generate the $[nM-nH+(n+1)Na]^+$ cluster ions. This may be due to the big sulfur atom. It is very hard that the (M−H+Na) of cysteine has a stable 6-membered ring since a sulfur atom is very big relative to an oxygen atom. If the (M−H+Na) of cysteine is formed, it may be a linear structure so that it can not play a role as a bidentate ligand.

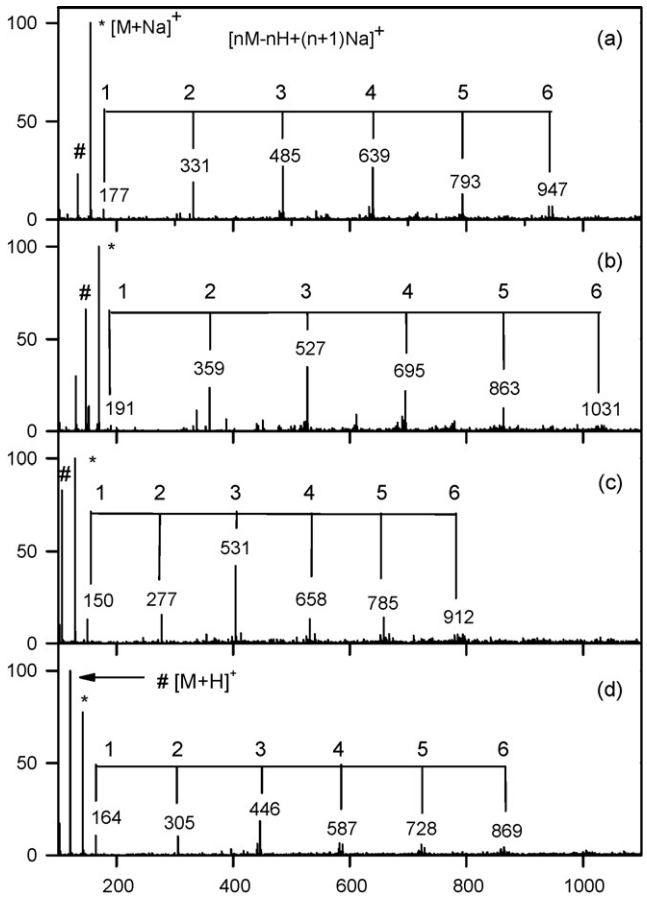


Fig. 1. Mass spectra of mixture solution of 1 mM NaOH+5 mM amino acid of asparagine (a), glutamine (b), serine (c), and threonine (d) at fragmentor voltage of 50 V. The numbers marked stand for the cluster size, n of the $[nM-nH+(n+1)Na]^+$. Asterisks (*) and sharps (#) indicate the $[M+Na]^+$ and $[M+H]^+$, respectively.

In order to investigate the cluster ion distributions in detail, the ion intensities of the $[nM-nH+(n+1)Na]^+$ ions were normalized with the ion intensity of the $[M+H]^+$ and the ion intensity ratios were plotted as a function of the cluster size as shown in Figs. 2–5. The relative cluster ion distributions of

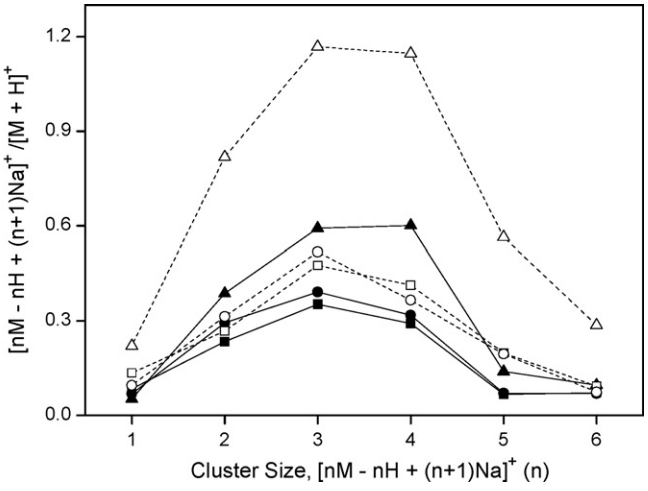
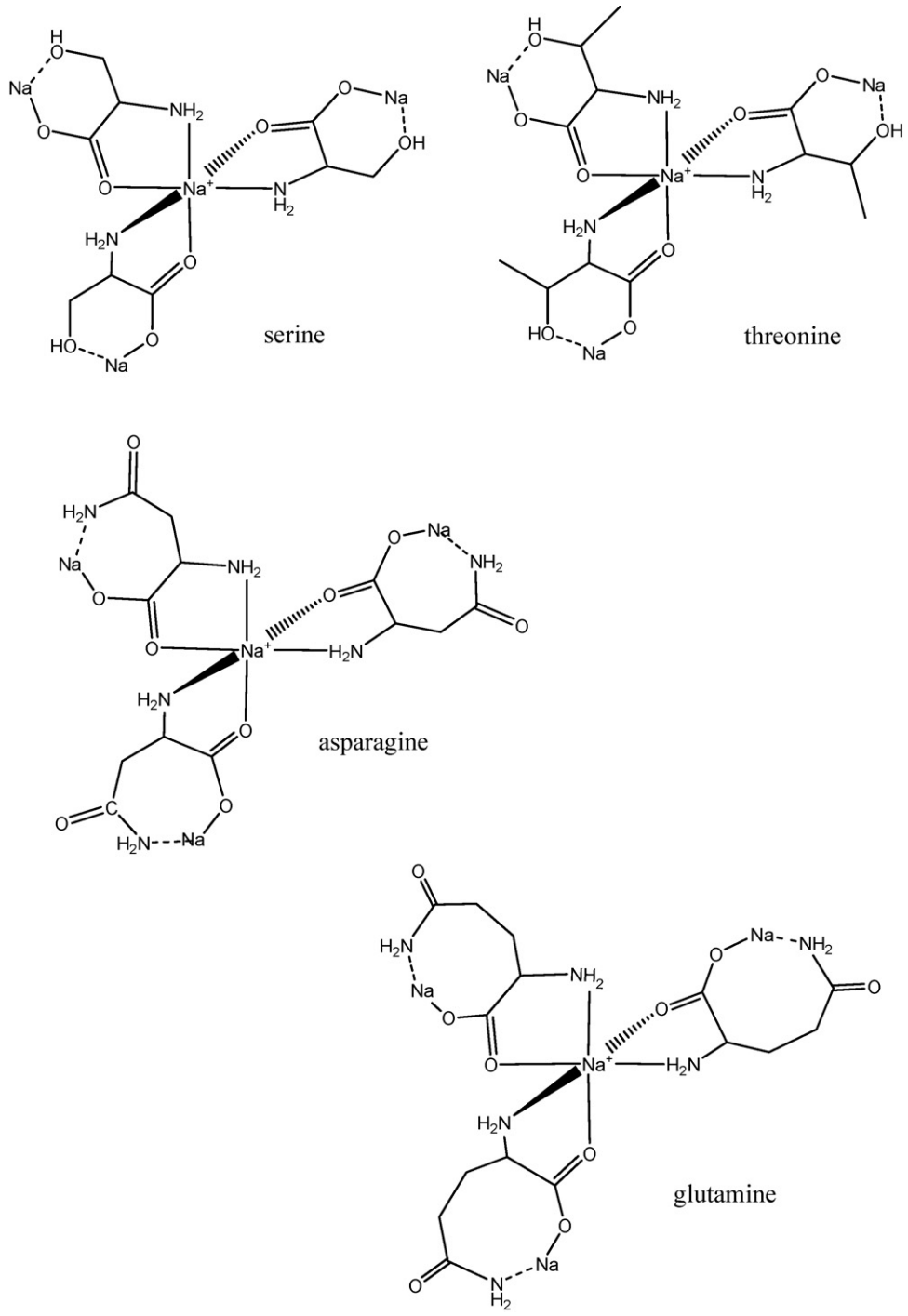


Fig. 2. Variation of the ion intensity ratio of $[nM-nH+(n+1)Na]^+/[M+H]^+$ of asparagine with the cluster size. Squares, circles, and triangles stand for the 0.25, 0.5, and 1 mM NaOH solutions, respectively. Solid and open symbols indicate the fragmentor voltages of 30 and 50 V, respectively.



Scheme 1. Plausible structures of $[3M-3H+4Na]^+$ cluster ions of asparagine, glutamine, serine, and threonine.

$[nM-nH+(n+1)Na]^+/[M+H]^+$ showed a steep decrease in the ion intensity following cluster ions referred to as a magic number. The magic number means that the species of $n=3$ is more stable than the others. We can suggest that the $[3M-3H+4Na]^+$ ions have six-coordinated structures as shown in Scheme 1. Two polar groups of ketone and amine in the proton-sodium cation exchanged species can bind to the centered sodium cation. Thus, the proton-sodium cation exchanged species can play a role of bidentate ligand. The (M−H+Na) molecules of asparagine, glutamine, serine, and threonine can have 7-, 8-, 6-, and 6-membered cyclic structures, respectively, as shown in Scheme 1. For serine and threonine, the sodium atom (or cation) in the (M−H+Na) molecule is stabilized by hydrogen bonding with terminal hydroxyl group. For asparagine and glutamine, the sodium atom (or cation) can be stabilized by hydrogen bonding with terminal amine group. Thus, the sodium complex, $(M-H+Na)_3Na^+$ has a stable hexadentated structure.

Fig. 2 displayed the cluster ion distributions of asparagine. The relative ion intensity ratios of $[nM-nH+(n+1)Na]^+/[M+H]^+$ at the high NaOH concentration of 1.0 mM are much higher than those at the low NaOH concentrations of 0.25 and 0.50 mM. In addition, the $[nM-nH+(n+1)Na]^+/[M+H]^+$ ratios at the high fragmentor voltage of 50 V are larger than those at the low fragmentor voltage of 30 V.

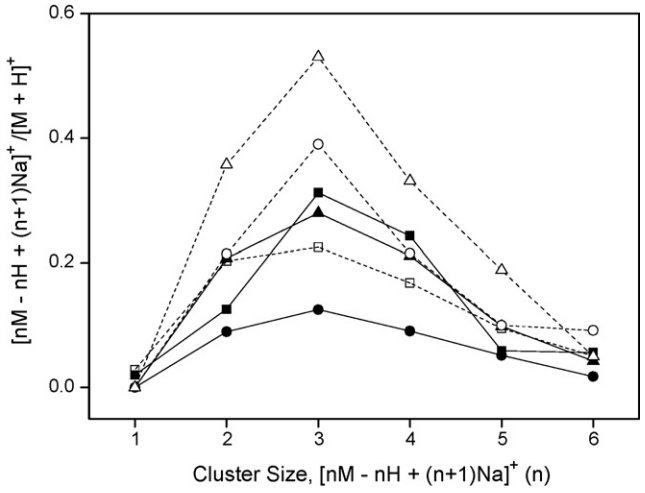


Fig. 3. Variation of the ion intensity ratio of $[n\mathrm{M}-n\mathrm{H}+(n+1)\mathrm{Na}]^+/[\mathrm{M}+\mathrm{H}]^+$ of glutamine with the cluster size. Squares, circles, and triangles stand for the 0.25, 0.5, and 1 mM NaOH solutions, respectively. Solid and open symbols indicate the fragmentor voltages of 30 and 50 V, respectively.

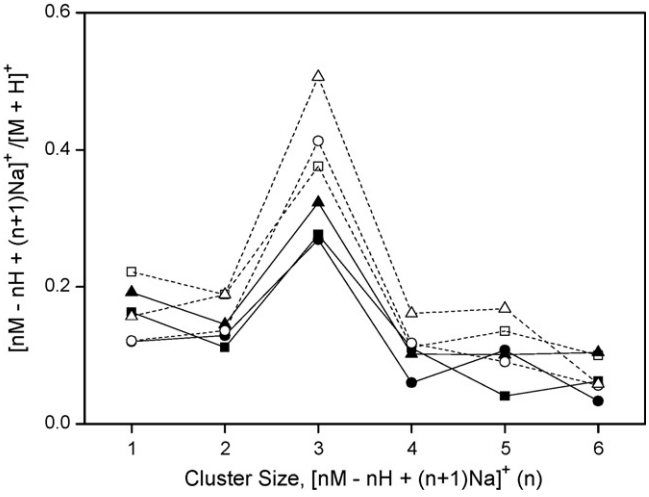


Fig. 4. Variation of the ion intensity ratio of $[n\mathrm{M}-n\mathrm{H}+(n+1)\mathrm{Na}]^+/[\mathrm{M}+\mathrm{H}]^+$ of serine with the cluster size. Squares, circles, and triangles stand for the 0.25, 0.5, and 1 mM NaOH solutions, respectively. Solid and open symbols indicate the fragmentor voltages of 30 and 50 V, respectively.

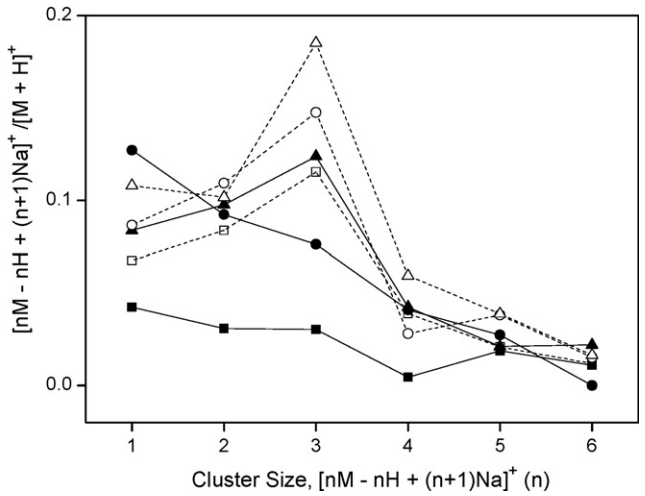


Fig. 5. Variation of the ion intensity ratio of $[n\mathrm{M}-n\mathrm{H}+(n+1)\mathrm{Na}]^+/[\mathrm{M}+\mathrm{H}]^+$ of threonine with the cluster size. Squares, circles, and triangles stand for the 0.25, 0.5, and 1 mM NaOH solutions, respectively. Solid and open symbols indicate the fragmentor voltages of 30 and 50 V, respectively.

At only the low fragmentor voltage of 30 V and high NaOH concentration of 1.0 mM, the magic number reached $n = 4$ instead of 3. This may be because numerous (M−H+Na) molecules were generated at sufficiently high sodium cation concentration, thus the equilibrium shifted to larger cluster size. The cluster ion distributions of glutamine also had a magic number of $n = 3$ as shown in Fig. 3. This whole trend was similar to what was noted for asparagine. The relative ion intensity ratios of $[n\mathrm{M}-n\mathrm{H}+(n+1)\mathrm{Na}]^+/[\mathrm{M}+\mathrm{H}]^+$ of glutamine were smaller than those of asparagine. This can be explained by the ion intensities of the $[\mathrm{M}+\mathrm{H}]^+$ ions. The relative ion intensity of the $[\text{glutamine}+\mathrm{H}]^+$ was higher than that of the $[\text{asparagine}+\mathrm{H}]^+$ as shown in Fig. 1.

The plausible structures of the proton-sodium cation exchanged molecules of serine and threonine are 6-membered rings (Scheme 1). The cluster ion distributions of serine most clearly displayed the magic number of $n = 3$ as shown in Fig. 4. The relative ion intensity ratios of $[3\mathrm{M}-3\mathrm{H}+4\mathrm{Na}]^+/[\mathrm{M}+\mathrm{H}]^+$ for serine were similar to the glutamine though the relative ion intensity of the $[\text{serine}+\mathrm{H}]^+$ was larger than that of the $[\text{glutamine}+\mathrm{H}]^+$ as shown in Fig. 1. There was no significant change in the clearance of the magic number at the fragmentor voltages of 30 and 50 V. This indicates that the $[3\mathrm{M}-3\mathrm{H}+4\mathrm{Na}]^+$ of serine has a relatively more favorable structure. The other three amino acids did not show this phenomenon. This may be due to the size of the (M−H+Na) molecule. Since the (M−H+Na) of serine is smaller than the others, the $[3\mathrm{M}-3\mathrm{H}+4\mathrm{Na}]^+$ of serine is less sterically hindered than the others. The cluster ion distributions of threonine showed some different trends as shown in Fig. 5. The cluster ion distributions of threonine at the fragmentor voltage of 50 V showed the magic number, but those at the fragmentor voltage of 30 V and the NaOH concentrations of 0.25 and 0.50 mM did not display the magic number. The relative ion intensity ratios of $[3\mathrm{M}-3\mathrm{H}+4\mathrm{Na}]^+/[\mathrm{M}+\mathrm{H}]^+$ of threonine at the fragmentor voltage of 30 V and the NaOH concentrations of 0.25 and 0.50 mM decreased with the increases in the cluster size.

Asparagine and glutamine generated the cluster ions, but aspartic acid and glutamic acid did not produce their cluster ions. This may be because aspartic acid and glutamic acid are composed of γ-CO_2H so the proton-sodium cation exchanged molecules will exist in the form of zwitterions through proton transfer of the γ-CO_2H to the amine group to form the ammonium ion ($-NH_3^+$) which can not play role of a ligand. Thus, zwitterion cannot play a role of a bidentate ligand.

4. Conclusions

Twenty proteinic amino acid solutions containing Na^+ were ionized by electrospray and the product ions were analyzed. They commonly generated $[\mathrm{M}+\mathrm{H}]^+$ and $[\mathrm{M}+\mathrm{Na}]^+$. Only asparagine, glutamine, serine, and threonine generated the cluster ions of $[n\mathrm{M}-n\mathrm{H}+(n+1)\mathrm{Na}]^+$ which are sodium-cationized clusters of (M−H+Na). The neutral (M−H+Na) molecule was the proton-sodium cation exchanged species which can develop 7-, 8-, 6-, and 6-membered cyclic structures for asparagine, glutamine, serine, and threonine, respectively. The cluster ion distributions displayed the magic number of $n = 3$. The $[3\mathrm{M}-3\mathrm{H}+4\mathrm{Na}]^+$ cluster ion had a six-coordinated structure of $(\mathrm{M}-\mathrm{H}+\mathrm{Na})_3\mathrm{Na}^+$ and the neutral (M−H+Na) molecule played a role of a bidentate ligand. The magic number was clearer at the fragmentor voltage of 50 V than at 30 V. The magic number for the cluster ion distributions of serine was clearer than the others.

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