

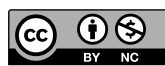
Original Article

Gas-Phase Adsorption of N₂ on Protonated Molecules and Its Application to the Structural Elucidation of Small Molecules

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The gas-phase adsorption of N₂ on protonated serine (Ser, C₃H₇NO₃), threonine (Thr, C₄H₉NO₃), glycine (Gly, C₂H₅NO₂), and 2-aminoethanol (C₂H₇NO) was investigated using a tandem mass spectrometer equipped with an electrospray ionization source and a cold ion trap. N₂ molecules were adsorbed on the free X–H (X=O and N) groups of protonated molecules. Gas-phase N₂ adsorption-mass spectrometry detected the presence of free X–H groups in the molecular structures, and was applied to the structural elucidation of small molecules. When the 93 structures with an elemental composition of C₃H₇NO₃ were filtered using the gas-phase N₂ adsorption-mass spectrometry results for Ser, the number of possible molecular structures was reduced to 8 *via* the quantification of the X–H groups. Restricting and minimizing the number of possible candidates were effective steps in the structural elucidation process. Gas-phase N₂ adsorption-mass spectrometry combined with mass spectrometry-based techniques has the potential for being useful for elucidating the molecular structures of a variety of molecules.



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INTRODUCTION

Molecular structures are typically identified using nuclear magnetic resonance spectroscopy, X-ray crystallography, optical spectroscopy, and chromatography. Mass spectrometry is routinely used for structural elucidation owing to its high sensitivity, selectivity, and suitability for analyzing mixtures. The elemental compositions of unknown molecules are assigned using high-resolution and accurate mass spectra collected for complex mixtures.¹⁾ The number of potential elemental compositions, which increases largely with mass, can be reduced by comparing the experimental and theoretical isotope patterns in mass spectra.²⁾

When the elemental composition of an analyte is elucidated using mass spectrometry, the molecular structure is examined utilizing product ion profiles that are obtained *via* tandem mass spectrometry. The relationships between molecular structure and fragmentation have been studied using a variety of dissociation techniques.^{3,4)} Ion mobility and hydrogen/deuterium exchange have been used to analyze molecular structures.^{5,6)} Nuclear magnetic resonance spectroscopy of gas-phase ions using a magnetic resonance acceleration technique has been developed.⁷⁾ Mass spectral libraries and electronic databases are typically surveyed for

the structural elucidation of unknown molecules in complex mixtures, because the number of potential molecular structures with any elemental composition increases exponentially with mass.^{8–10)}

The molecular adsorption of gas-phase ions provides information on the structure of gas-phase ions. N₂ adsorption on small metal clusters of cations depends on the structure of the cations.^{11,12)} Molecular adsorption experiments on gas-phase carbohydrate ions with 2–5 hydroxy (O–H) groups revealed that the O–H groups of carbohydrates could be quantified using gas-phase N₂ adsorption-mass spectrometry.¹³⁾

In this study, we investigated the adsorption of gas-phase N₂ on protonated molecules using a tandem mass spectrometer equipped with an electrospray ionization source and temperature-controlled ion trap. Gas-phase N₂ adsorption-mass spectrometry was used for structural elucidation in addition to assigning elemental composition and structural elucidation using fragmentation and mass spectral library surveys with serine (Ser, C₃H₇NO₃), threonine (Thr, C₄H₉NO₃), glycine (Gly, C₂H₅NO₂), and 2-aminoethanol (C₂H₇NO) being used as sample molecules.

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EXPERIMENTAL

Gas-phase N₂ adsorption was detected using a home-built tandem mass spectrometer equipped with an electrospray ionization source and a temperature-controlled 22-pole ion trap (8–350 K).^{14,15} Protonated molecules were generated *via* the electrospray ionization of solutions containing 0.5 mM of an analyte molecule in a mixture of water and methanol with 1% acetic acid. Analyte molecules were obtained from Nacalai Tesque. The ionization source was operated at a sample flow rate of 3 μL/min, a sheath gas flow rate of 3 L/min, and an applied voltage of 1.5 kV. The protonated molecules were transferred to the gas phase through a metal capillary (340 K) and stored in an octupole ion guide. The gas-phase ions were pulsed into a quadrupole mass filter and a temperature-controlled 22-pole ion trap. The mass-selected ions were thermalized *via* multiple collisions with He containing 30% N₂ as the buffer gas for 50 ms in the ion trap at 60 K, and the N₂ molecules were adsorbed on the mass-selected ions in the ion trap. At lower temperatures, the N₂ molecules condensed on the ion trap electrodes, while at room temperature, the N₂ molecules were not adsorbed on the ions. The temperature of the ion trap was controlled using a cryogenic refrigerator (CH-204SB, Sumitomo, Japan) and a heater cartridge (HTR-50, Lake Shore, USA), and measured using two silicon diode temperature sensors (DT-670B-CU, Lake Shore, USA). The exact partial pressure and temperature of the N₂ molecules in the ion trap were not determined in this study, and the ambient pressure in the chamber containing the ion trap was approximately 2 × 10⁻⁴ Pa. The maximum numbers of N₂ molecules that were adsorbed did not depend on the chamber pressure, but the efficiency of N₂ adsorption was dependent on this. The N₂-adsorbed cluster ions were extracted from the ion trap and orthogonally accelerated to 2.8 keV using two-stage pulsed electric fields. The ions were mass-analyzed using a reflectron time-of-flight (TOF) spectrometer and counted using dual microchannel plates (F4655, Hamamatsu Photonics, Japan) and a digital storage oscilloscope (104MXi, LeCroy, Japan). The desorption of N₂ from the weakly bound clusters in the field-free region of the reflectron TOF mass spectrometer was also observed in the TOF mass spectra, in addition to the N₂-adsorbed cluster ions that were formed in the ion trap.¹⁵ The repetition rate of the experimental cycle was 10 Hz.

RESULTS AND DISCUSSION

Gas-phase adsorption of N₂ on protonated molecules

Figure 1a illustrates the mass spectrum of H⁺Ser(N₂)_n, which formed *via* the adsorption of N₂ on mass-selected H⁺Ser at 60 K. Here, *n* denotes the number of N₂ molecules that are adsorbed on the H⁺Ser in the ion trap, which, according to our experimental results, was *n* ≤ 5. This indicates that the first solvation shell of H⁺Ser consisted of a maximum of five molecules. Stable magic number clusters formed *via* gas-phase N₂ adsorption on mass-selected ions were observed for carbohydrates and tryptophan (Trp).^{13,14} It was reported that N₂ molecules are adsorbed on the H atoms of the O–H groups of carbohydrates, which allowed for the O–H groups of carbohydrates to be quantified using

gas-phase N₂ adsorption data.¹³ H⁺Trp(N₂)₅ was a stable magic number cluster in which five N₂ molecules were adsorbed on the H atoms of the NH₃⁺ group, the indole ring, and the carboxyl (COOH) group.¹⁴ H⁺Trp(H₂)_n with *n* = 1–5 were reported to be formed in messenger-tagging vibrational predissociation spectroscopic measurements of H⁺Trp using a cryogenic ion trap.¹⁶ Diatomic molecules were primarily bound by charge-induced dipole interactions. The optimized structure of H⁺Ser(N₂)₅ at the B3LYP/6-31++G(d,p) level using the Gaussian 09 package¹⁷ was obtained utilizing previously reported data^{14,16,18} (Fig. 1b), and the results suggested that five N₂ molecules were adsorbed on the NH₃⁺, O–H, and COOH groups of the H⁺Ser. The relative ion intensity of H⁺Ser(N₂)₅ was lower than that of H⁺Ser(N₂)₄, as illustrated in Fig. 1a. The ion intensity distributions of the N₂-adsorbed cluster ions indicated that the gas-phase ions had multiple structures.¹³ The lower relative ion intensity of the *n* = 5 cluster was attributed to the intramolecular hydrogen bond between the NH₃⁺ group and the oxygen atom of COOH group as illustrated in Fig. 1b. In the case of Thr, like Ser, contains an O–H group, the maximum number of adsorbed N₂ molecules was *n* = 5 as illustrated in Fig. 2, indicating that the adsorption sites of H⁺Thr were the NH₃⁺, O–H, and COOH groups as in the case of H⁺Ser. To reveal the effects of molecular adsorption on the geometric and electronic structures of gas-phase ions, photodissociation spectroscopy and theoretical calculations are required.

To confirm the relationship between N₂ adsorption and molecular structure, gas-phase N₂ adsorption-mass spectrometry of H⁺Gly and H⁺(2-aminoethanol) was performed. Gly and 2-aminoethanol lack the O–H and COOH groups of Ser, respectively. Figure 3a illustrates the mass spectrum

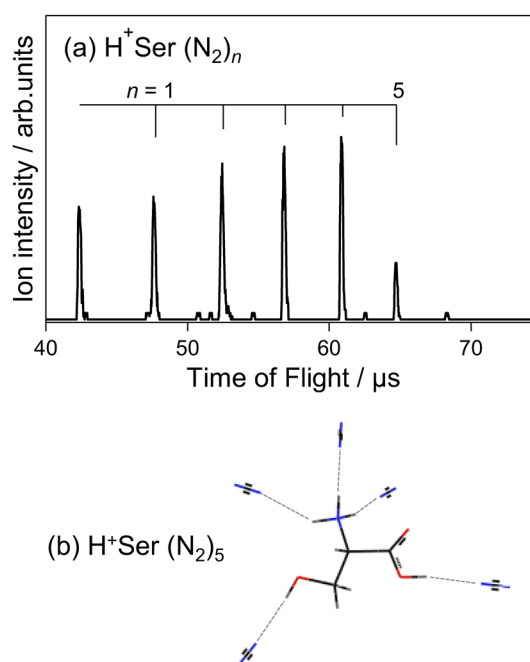


Fig. 1. (a) Mass spectrum of H⁺Ser(N₂)_n, formed *via* N₂ adsorption on mass-selected H⁺Ser at 60 K. (b) Optimized structure of H⁺Ser(N₂)₅ at the B3LYP/6-31++G(d,p) level, where carbon, nitrogen, oxygen, and hydrogen atoms are colored in black, blue, red, and gray, respectively.

of H⁺Gly(N₂)_n, which forms *via* the adsorption of N₂ on mass-selected H⁺Gly at 60 K. The maximum number of adsorbed N₂ molecules was *n*=4, and an *n*=5 cluster was not observed in the spectrum. The number of adsorption sites for H⁺Gly was one less than that of H⁺Ser and H⁺Thr. This indicates that four N₂ molecules were adsorbed on the NH₃⁺ and COOH groups of H⁺Gly. The maximum number of adsorbed N₂ molecules for H⁺(2-aminoethanol)(N₂)_n was also *n*=4 (Fig. 3b), indicating that four N₂ molecules were adsorbed on the NH₃⁺ and O-H groups of H⁺(2-aminoethanol). The above findings indicate that gas-phase N₂ adsorption-mass spectrometry can be used to detect the presence of free X-H (X=O and N) groups in molecular structures.

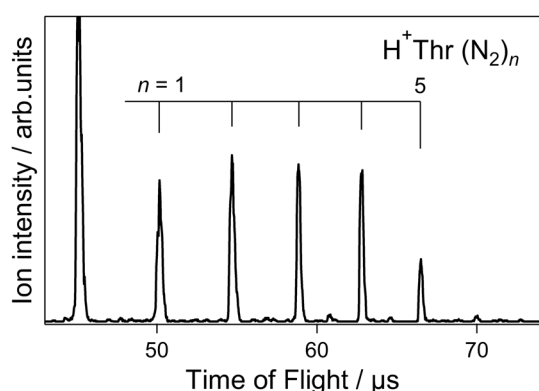


Fig. 2. Mass spectrum of H⁺Thr(N₂)_n, formed *via* the adsorption of N₂ on mass-selected H⁺Thr at 60 K.

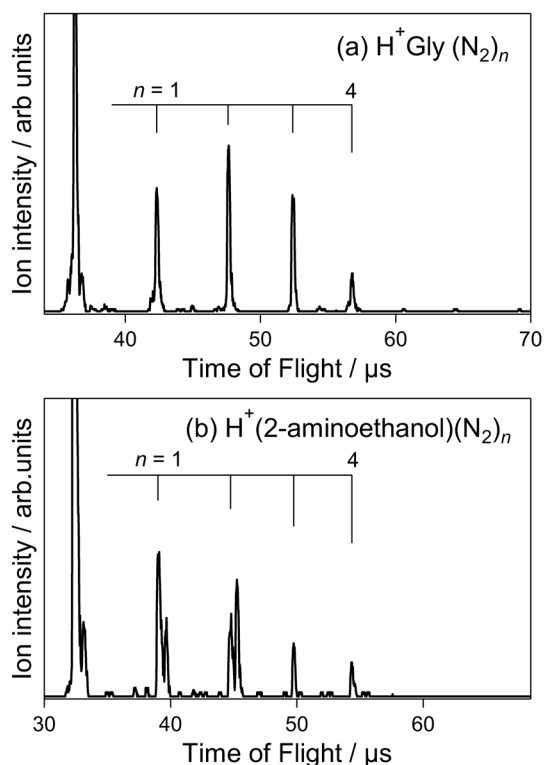


Fig. 3. Mass spectra of (a) H⁺Gly(N₂)_n and (b) H⁺(2-aminoethanol)(N₂)_n formed *via* the adsorption of N₂ on mass-selected H⁺Gly and H⁺(2-aminoethanol) at 60 K, respectively.

Structural elucidation using gas-phase N₂ adsorption-mass spectrometry

Based on the aforementioned results, we proposed a method for structural elucidation using gas-phase N₂ adsorption-mass spectrometry that can be used prior to fragmentation and in surveying mass spectral libraries. Figure 4 illustrates the strategy used for the structural characterization of Ser (C₃H₇NO₃, calculated exact mass 105.0426). The CAS registry revealed 93 possible structures with an empirical formula of C₃H₇NO₃. When conventional methods are used for characterizing molecular structures, the product ion spectrum of the protonated molecule is compared with the product ion spectra in a mass spectral library, and the structures that match the elemental composition and fragmentations are screened.⁸⁻¹⁰ Restricting and minimizing the number of possible candidates are effective steps of the analysis process.

Gas-phase N₂ adsorption-mass spectrometry can reduce the number of possible molecular structures with the same elemental composition *via* the quantification of the X-H (X=O and N) groups of the protonated molecule. For the C₃H₇NO₃ elemental composition, the maximum number of N₂ molecules adsorbed on the protonated molecule was *n*=5, which indicated that C₃H₇NO₃ contained four X-H groups. The number of adsorption sites of C₃H₇NO₃ was

Elemental composition assignment

using the mass spectra of protonated molecules

$$m/z \ 106.0504 \rightarrow \text{H}^+(\text{C}_3\text{H}_7\text{NO}_3)$$

$$105.0426 \text{ Da } \text{C}_3\text{H}_7\text{NO}_3 \rightarrow 93 \text{ structures}$$

Reduction in the number of molecular structures using gas-phase N₂ adsorption-mass spectrometry

$$\text{H}^+(\text{C}_3\text{H}_7\text{NO}_3)(\text{N}_2)_n \quad n \leq 5$$

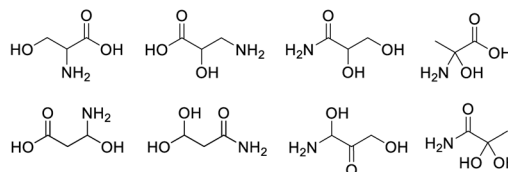
$$4 \text{ free OH and NH in } \text{C}_3\text{H}_7\text{NO}_3 \rightarrow 8 \text{ structures}$$

Identification of molecules

using messenger-tagging vibrational spectroscopy, chiral recognition of tryptophan, fragmentation, etc.

Fig. 4. Strategy for elucidating molecular structures using gas-phase N₂ adsorption-mass spectrometry.

(a) 4 X-H (X = O and N) groups for C₃H₇NO₃



(b) 3 X-H (X = O and N) groups for C₂H₅NO₂

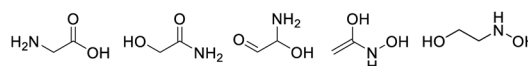


Fig. 5. Potential molecular structures with empirical structures of (a) C₃H₇NO₃ and (b) C₂H₅NO₂.

derived by subtracting of 1 from the maximum number $n=5$ of H⁺(C₃H₇NO₃), because one N₂ molecule adsorbed the proton. When the 93 structures associated with the elemental composition of C₃H₇NO₃ were filtered using the number of adsorption sites for C₃H₇NO₃, the number of possible molecular structures was reduced to eight (Fig. 5a). In the case of Gly (C₂H₅NO₂, calculated exact mass 75.0320), the 46 possible structures for C₂H₅NO₂ were reduced to 5 (Fig. 5b) *via* the quantification of the adsorption sites. This method decreased to 8.6 and 10.8% of the number of possible candidates obtained from the conventional methods for C₃H₇NO₃ and C₂H₅NO₂, respectively.

CONCLUSION

The gas-phase adsorption of N₂ on protonated molecules generated *via* electrospray ionization was investigated using a tandem mass spectrometer equipped with a temperature-controlled ion trap. This method allowed the number of possible molecular structures with the same elemental composition to be reduced by tenth *via* the quantification of the X–H (X=O and N) groups. Restricting the number of possible candidates is an effective step in the structural elucidation process.

The weakly bound clusters of analyte ions and N₂ formed in cold ion traps, such as H⁺(C₃H₇NO₃)(N₂), can be used for the messenger-tagging vibrational spectroscopy analysis of gas-phase analyte ions.¹⁹ The optical properties of Trp hydrogen-bonded with analyte ions in the gas phase can be used to identify and quantify the isomers and enantiomers of analyte molecules.^{20,21} An ion mobility spectrometer with radio-frequency electric fields has been developed for high-resolution and high-transmission efficiency analyses of small molecules.²² The findings reported herein indicate that gas-phase N₂ adsorption-mass spectrometry combined with mass spectrometry-based techniques can be useful for elucidating molecular structures.

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