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High-energy electron transfer dissociation of protonated amino acids

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ABSTRACT

High-energy electron transfer dissociation (HE-ETD) of singly protonated amino acids was investigated using charge inversion mass spectrometry with alkali metal targets in which dissociations of charge-reduced neutrals were induced from the excited neutral intermediates. The dominant trapezoidal peaks of the 17 u loss in the charge inversion spectra of the protonated aliphatic amino acids were assigned to NH_3 loss caused by N—Cα bond cleavage from hypervalent ammonium radical intermediates via a repulsive state; this assignment was based on the peak shape and kinetic energy release (KER) values. The peak of the non-dissociated ion observed in the proline spectra was explained by the same bond cleavage. The triangular peaks of the 2 u loss, which were dominantly observed in all of the spectra, were assigned to H_2 elimination, which involved H atom migration in the neutral radical. Neutral intermediates that induced the H_2 loss were estimated to have a lower internal energy than that of the NH_3 loss by comparing the charge inversion spectra of the different target atoms. Differences in the isobaric ions between leucine and isoleucine were found in the bond dissociation of Cβ—Cγ in their side chains following the NH_3 loss and in the intensity of the NH_3 loss relative to that of the H_2 loss. The charge inversion spectra of the protonated amino acids, with side chains that are subject to post-translational modification, provided intense peaks resulting from the consecutive and competitive loss of neutral molecules following the NH_3 loss and H_2 loss. Along with these losses, side chain losses caused by H atom migration were also observed as sharp peaks with KER values smaller than that of the consecutive loss.

1. Introduction

Development of the so-called soft-ionization methods, such as electrospray ionization (ESI) [1] and matrix-assisted laser desorption/ionization (MALDI) [2,3] enables the use of mass spectrometry to analyze biomolecules, such as peptides and proteins. These ionization methods do not produce the radical cation of the molecules, instead producing the protonated molecules of the even electron. For structure analysis of the gaseous ions including the protonated molecules, collisionally activated dissociation (CAD) has been used as a traditional method in various tandem mass spectrometers (MS/MS) [4] on the market. The disadvantages of CAD, which does not provide comprehensive backbone cleavage for the protein and peptide, especially when they include the proline residue [5,6], and does not provide the position of the post translational group [7], have been overcome by electron capture dissociation (ECD) [8] equipped in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) and electron transfer dissociation (ETD) [9,10] equipped in an ion trap mass spectrometer. ETD of protonated peptides and proteins is rich and diverse for sequence elucidation, especially for determining the position of the post-translational modification. The mechanism of ECD and ETD has been discussed extensively [11–14].

ECD or ETD measurement using small molecules is useful for the elucidation of the ETD mechanism. In contrast to polypeptides, amino acids, except for basic amino acids, provide only singly charged protonated molecules. Because electron capture or electron transfer to singly charged ions produces neutral radicals, which cannot be controlled and analyzed by the electromagnetic field used in mass spectrometers, ECD and ETD cannot be employed in the analysis of the singly protonated molecules. Detection methods applicable to high-energy neutral fragments for simple molecules have been developed by Porter et al. [15–17] and Sidis et al. [18]. The dissociation mechanism of hypervalent neutral intermediates formed from protonated molecules by electron transfer from alkali metal targets was reported using the methods by Porter et al. [15–17]. The backbone cleavage of doubly protonated peptide by electron transfer dissociation from a Na target was first reported by the Aarhus group [19]. By the same group, dissociation mechanisms of charge reduced protonated di- and tri-peptides, some of

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which were partially deuterium or ^{15}N-labeling, were also investigated by using a charge reversal experiment using alkali metal targets [20–24].

To investigate the dissociation mechanism of various neutrals, many types of neutralization–reionization mass spectrometry [25–30] were developed in which the neutralization step and reionization step were separated using two collision cells. Especially, neutralization of N-protonated molecules including NH_4^+ provides the hypervalent ammonium radical [31–35]. The hypervalent ammonium radical produced by electron capture in protonated peptides is one of the transient intermediates that induces the dissociation of protein and peptides in ECD and ETD. Competitive elimination of ammonia, H-atom loss, and H-atom migration to neighboring amide carbonyls from the hypervalent ammonium radicals were discussed based on an NRMS experiment and the quantum chemical calculation by the Aarhus group [23,24] and Turecek and coworkers [31–33,35].

As a type of neutralization reionization method, an alternative method of forming the excited neutral species from the singly charged cation has been developed using electron transfer processes from positive ions in charge inversion mass spectrometry [36,37]. In charge inversion mass spectrometry, positive ions are forced to collide with an alkali metal target, and the resulting negative ions formed upon the two-electron transfer are mass analyzed. Using thermometer molecules, it has been demonstrated that the process of dissociative negative ion formation occurs via near-resonant neutralization (reaction (1)), followed by spontaneous dissociation of the formed neutral (reaction (2)), and then endothermic negative ion formation (reaction (3)), as given in the following scheme [36,38,39]:

$$AB^+ + T \rightarrow AB^* + T^+ \quad (1)$$

$$AB^* \rightarrow A + B \quad (2)$$

and

$$A(\text{or } B) + T \rightarrow A^-(\text{or } B^-) + T^+ \quad (3)$$

Using this technique, unstable neutral intermediates, such as vinylidenes [40,41], CH_n (n = 3–5) [42], CH_2X_2 (X = Cl, Br, and I) [43,44], and CH_3X (X = Cl, Br, and I) [45], have been studied. Protonated molecules $[AB+H]^+$, which are positive ions formed from stable molecules (AB), are in a singlet state (as are stable molecules) because the proton does not have an electron associated with it. Because target alkali metals are in doublet states, the spin multiplicity of the neutral intermediates (AB^*) formed by an electron transfer can be in a doublet state. Therefore, charge inversion mass spectrometry allows the examination of the dissociation dynamics of the neutral intermediate in the doublet state. HE-ETD for the protonated dipeptide including a proline residue [46] and basic amino acids [47,48] were previously discussed along with the quantum chemical calculation.

In the present study, we measured the charge inversion spectra of protonated amino acids using Na, K, and Cs targets. Glycine, alanine, leucine, isoleucine, proline, serine, threonine, and cysteine are selected as the amino acids whose protonated site is expected to be the N-terminal [49–51] and whose doubly protonated species cannot be formed. The internal energies of neutral species formed by electron transfer are varied by changing the target alkali metals (Na, K, and Cs). From the relative intensities of the fragment ions and the kinetic energy release (KER) values evaluated from the peak widths [52–57], the dissociation process of the neutral intermediates formed from the corresponding protonated molecules by neutralization is discussed. It is revealed that the consecutive and competitive dissociations are dependent on the amino acids used.

2. Experimental

The MS/MS instrument used in this study is composed of a double-focusing mass spectrometer (Hitachi M80B) as MS-I to mass separate the precursor ions, a 4 cm long collision cell, and a toroidal electrostatic analyzer with a 216 mm central radius as MS-II to mass analyze the secondary ions [36]. Amino acids were introduced into the ionization chamber by a direct inlet probe and were protonated by chemical ionization (CI) with isobutane as the reagent gas. The formed ions were accelerated to 2970 eV, mass selected by MS-I, and focused on a collision cell filled with alkali metal vapor. The alkali metal target was supplied from a reservoir through a ball valve. By closing the ball valve, supply of the alkali metal target was easily cut off. Precursor ion spectra were measured under the conditions of no alkali metal target. Charge inversion mass spectra were measured by mass analyzing the negative ions exiting the collision cell only by altering the polarity of MS-II and the detector. The detector was a 10 kV post-acceleration secondary electron multiplier, which could detect the positive and negative ions upon application of a suitable polarity. In the charge inversion process, neutralization, dissociation, and negative ion formation occurred in the collision cell. By setting the width of the focusing slit in front of the collision cell to 200 μm, mass analysis of the precursor ions provided a high resolution by double focusing the mass spectrometer of MS-I. Because the width of entrance and the exit slit of the collision cell are 1.0 mm and 2.0 mm, respectively, all of the fragment ions including those having a large transverse velocity by KER could enter the sector of the MS-II and were energy-analyzed under the conditions of angular focusing by MS-II. Using this analyzing system in MS-II, the mass analysis of the fragment ions did not provide a high resolution, but the KER values of the fragment ions were reliably evaluated from the peak widths.

Amino acids were purchased from Wako, Japan and were used as received. Isobutane (Takachiho, Japan) gas was also used as received.

3. Results and discussion

3.1. Dissociation processes in charge inversion mass spectra

Singly charged negative ions from singly charged positive ions by a charge inversion process can be formed by either successive single electron transfers in two collisions or double electron transfers in a single collision. When an alkali metal is used as a target, the double electron transfer in a single collision is a highly endothermic process because of the large ionization energy in doubly charged positive ions of alkali metal atoms, with values of 28.97 eV, 36.15 eV, and 52.43 eV for Cs, K, and Na, respectively [58]. On the other hand, neutralization processes in the successive single electron transfers are typically exothermic because of the small ionization energy required to form singly charged positive ions, with values of 3.893 eV, 4.339 eV, and 5.138 eV for Cs, K, and Na, respectively [58]. Negative ion formation in the successive single electron transfers is slightly endothermic because of the small electron affinities of organic molecules. Previous studies in which the target density dependences of the negative ions were measured indicated that most of the negative ions observed in charge-inversion mass spectra were formed by successive single electron transfers in two collisions, as shown in reactions (1)–(3) [36,59]. The electron transfer process provides a large cross section for near-resonant processes [60,61]. Because the neutralization process is highly exothermic, the charge-reduced neutral intermediates formed from the singly charged positive ions are highly excited by near-resonant neutralization, as shown in previous studies [36,38,39]. Therefore, dissociations in the charge inversion mass

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