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International Journal of Mass Spectrometry 225 (2003) 83–87

International Journal of Mass Spectrometry

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Electron capture induced dissociation of peptide dications

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Received 18 September 2002; accepted 17 October 2002

Abstract

Dissociation induced by electron capture in high energy collisions between doubly protonated peptide ions and Na atoms has been investigated. The ions were produced in an electrospray ion source and accelerated to an energy of 100 keV before they were excited in collisions with a Na target gas. Electron capture was found to be the dominant reaction channel but also fragment peaks corresponding to cleavage of the backbone N–C_α bonds the so called c and z ions are prominent in the recorded mass spectra. Electron capture dissociation (ECD) where free electrons are captured by ions stored in a FTICR cell has previously been shown to result in sequence information. Similar measurements have been performed in both a Ne and a Mg target and by comparing the mass spectra for the three target gases it is concluded that electron capture by protonated peptides in high energy collisions leads to non-ergodic fragmentation of the peptide ion.

Keywords: Electron capture; Dissociation; Peptides

1. Introduction

The aim of tandem mass spectrometry (MS/MS) [1,2] experiments is to obtain information about the structure of molecular ions. As an example amino acid sequence information of peptides is obtained if the peptide ions undergo reactions where all the bonds between the constituting amino acids are selectively cleaved with comparable probabilities in the individual reactions. Collision-induced dissociation (CID) [3,4] is the conventional sequencing technique which leads to vibrational excitation followed by intramolecular energy redistribution before fragmentation. The fragment spectra resulting from CID are normally dominated by b and y ions [5] resulting from cleavage of the amide bonds. The fragments produced in low energy CID only reflect cleavage of the lowest energy bonds and thus do not always give information about the full amino acid sequence. In high energy CID a broader range of fragments is observed. More fragments give more information but also lead to a larger complexity of the spectra and hence make the interpretation more difficult.

In an attempt to find the "ideal reaction" that leads to simple but informative fragment spectra, Zubarev and co-workers [6–8] invented what is now known as electron capture dissociation (ECD). In short this technique utilizes a Fourier transform mass spectrometer where the precursor ions are stored in a Penning ion trap and irradiated by thermal electrons. After electron–ion recombination the ions fragment and the dominant fragments are c and z ions stemming from the cleavage of the backbone N–C_α bond [7,9]. In

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1387-3806/02/$ – see front matter
PII S1387-3806(02)01039-4

contrast to CID, ECD is believed to be non-ergodic which means that cleavage occurs before the transferred energy is distributed over the entire molecule. ECD also occurs with a lesser dependence on the nature of the neighboring amino acid and only the N-side of proline is 100% resistive to ECD cleavage.

Electron capture induced fragmentation in collisions between molecular ions and gases has been studied by several groups [10–12]. Vékey et al. [10] studied as an example decomposition of the benzene dication $C_6H_6^{2+}$ and found that they could obtain information about the internal energy of the daughter ion by varying the ionization energy of the target. The case where a monocation captures two electrons from a target gas before it fragments belongs to a group of reactions dubbed charge permutation reactions. These reactions have been reviewed by Danell and Glish [12] and has mainly been applied to smaller molecules, but charge reversal ion spectra of polypeptides have provided extra sequence information in selected cases. Charge reversal mass spectrometry using alkali metal targets has been described by Hayakawa [13]. The molecular anions investigated are the so-called thermometer ions namely partially deuterated methanol and $W(CO)_6$. The results demonstrated that energy-selected neutrals can become candidates for isomeric differentiation.

In a previous paper [14] we have described how collisions between peptide or protein cations and Na or C_{60} targets lead to large electron capture cross-sections. The present work focuses on fragmentation of dications in high energy collisions with Ne, Na and Mg targets. It is demonstrated that the fragmentation pathways after collisions with Na or Mg where electron capture plays a dominant role also includes those found in ECD. It indicates that also electron capture by peptide ions in collisions with atoms with low ionization energies leads to non-ergodic fragmentation as does capture of free electrons. This finding was actually predicted by McLafferty [15] before ECD was invented. When Ne is used as target gas, the fragment spectrum is similar to those obtained in high energy CID. Based on a naive picture of Na as a Ne atom and a loosely bound electron we have subtracted the Ne spectrum from the Na spectrum and obtained a spectrum almost only consisting of a capture peak and peaks that can be associated with c ions. In this way a simple fragment spectrum that gives the full amino-acid sequence of the peptide in question is obtained. We suggest that this method could be considered as an attractive alternative to ECD in single pass experiments where a free electron target is difficult to implement.

2. Experiments

The experimental arrangement is described in detail in [16]. Briefly, doubly protonated peptide ions, formed by electrospray ionization (ESI), were accelerated by an electrostatic potential of 50 kV. The precursor ions were mass selected with a magnet and passed through a target cell. For the noble gas target Ne, we used a 3-cm long gas cell in which the gas pressure was monitored by a cold cathode gauge. For Na and Mg, the target cell was a resistively heated 6-cm long stainless-steel tube where the 4-cm long central part was defined by 1- and 2-mm-diameter entrance and exit apertures, respectively. Solid sodium or magnesium was placed in the mid section of the tube between the two apertures. During the experiment sodium was heated to temperatures around 200 °C and magnesium to 350 °C. The absolute target thickness was obtained from measured oven temperatures by use of vapor-vs.-temperature values [17]. The product ions exiting the cell were analyzed with an electrostatic hemispherical analyzer and fragmentation spectra were obtained.

3. Results and discussions

The fragment spectra recorded for doubly protonated amidated Substance P ions that have collided with Ne or Na atoms are shown in Fig. 1. Substance P is a 11-mer peptide (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met) which in its doubly protonated form has a mass of 1348 Da. It has been used in the

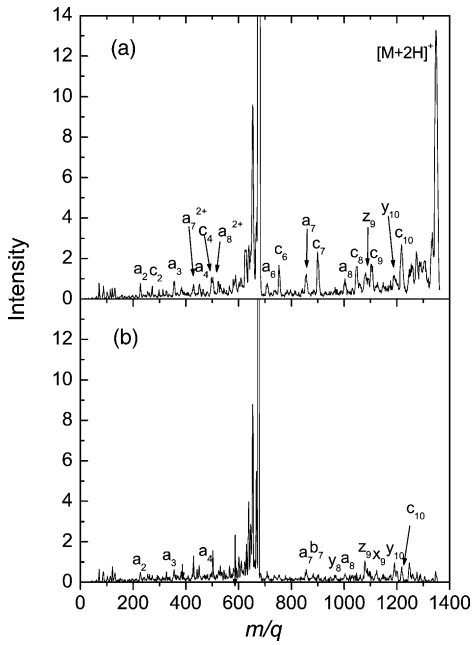

Fig. 1. CID spectra of Substance P obtained with Na (a) and Ne (b).

present investigations as a test case since its cleavage pattern has been previously studied by ECD [8,9]. The signal intensity in Fig. 1 is normalized to the target thickness and the intensity of the initial beam. It is clearly seen that the electron capture product peak, $[M + 2H]^{\bullet +}$ is the dominating peak in the Na spectrum while it is very weak in the Ne spectrum. The electron capture cross-section of doubly protonated Substance P has been measured in the Na target, and the cross-section for this electron capture reaction is found to be $\sim$13 Å^2. In both spectra peaks that are characteristic for high energy CID are observed with comparable intensities. It is however also clearly seen that there is a strong enhancement of peaks corresponding to breaks of the N–C_α bonds the so called c ions (c_6, c_7, c_8, c_9, c_{10}) in the Na spectrum, peaks that are the dominating ones in ECD spectra [7,8]. Since the mass of the two target atoms is quite similar the center of mass energies are almost identical. This would most probably result in similar fragmentation patterns if the conventional high energy CID process alone was responsible for bond cleavage. The

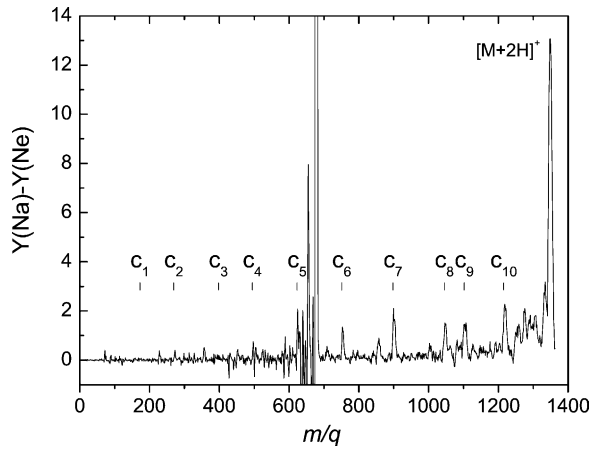

Fig. 2. Differences between two spectra of Substance P, the spectrum was obtained by subtracting spectrum with Ne from one with Na.

main difference between the two target gases is the ionization energy, which is 5.14 eV for Na while it is 21.56 eV for Ne [17]. This together with the finding of a doubly protonated ion that has captured an electron in Na but not in Ne suggests that the difference between the two cases is due to electron capture in the Na target. In Fig. 2 a difference spectrum is shown. This spectrum is very similar to the fragmentation spectrum obtained for Substance P in ECD. Mainly c ions are found which facilitates the sequence determination. However, the peaks corresponding to c_1 and c_3 are like in ECD missing. It is well-known that ECD is unable to break the amine backbone bonds on the N-terminal side of proline [7]. The predominance of N-terminal c ions can like in ECD be understood from the amino acid sequence of Substance P. The N-terminal amino acid is arginine that is likely to carry the charge since it is the most basic amino acid. The complementary z fragments are hence neutral and can not be detected in the present experiment.

The fragment spectra recorded for dications of a tryptic decapeptide from signal recognition particle (SRP) of *Saccharomyces cerevisiae* that have collided with Ne or Na atoms are shown in Fig. 3. The (SRP) peptide (Ser-Asp-Arg-Glu-Tyr-Pro-Leu-Leu-Ile-Arg) has in its doubly protonated form a mass of 1262 Da. Like for Substance P the two spectra show several

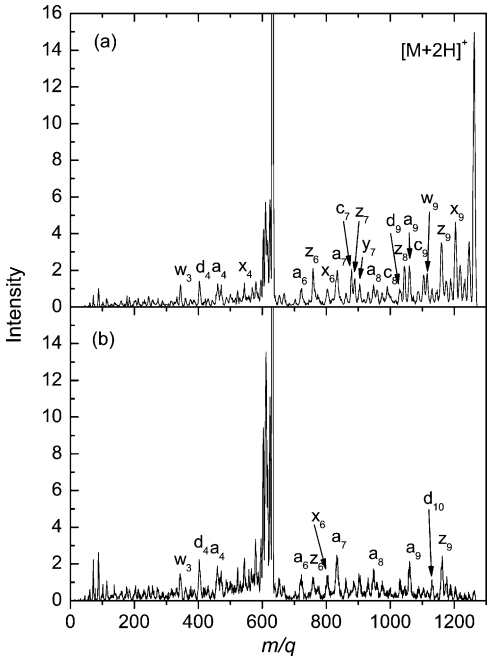

Fig. 3. CID spectra of SRP with Na (a) and Ne (b).

peaks that are characteristic for high energy CID spectra but again the electron capture product peak, $[M + 2H]^{\bullet +}$ is the dominating peak in the Na spectrum while it is very weak in the Ne spectrum. It should also be noted that several peaks which are characteristic for ECD spectra [18] are observed in the Na case. In contrast to the case of Substance P both c ions and their complementary z ions are observed. This can be explained by the presence of two arginine amino acids in the SRP peptide as nos. 3 and 10 one of which is most likely to carry the charge. In the ECD spectrum of SRP in [18] the dominating peaks correspond to z_6^+, c_7^+, c_9^+ and $[M + 2H]^{\bullet +}$ ions peaks that are also prominent in the Na spectrum in the present experiment. We also clearly observe peaks that can be related to z_9 and x_9 ions in the Na spectrum. These peaks are almost absent in the ECD spectrum in [18] a difference between ECD and our high energy electron capture-induced fragmentation measurements that it not understood at present.

It is also interesting to compare the present SRP Na spectrum with a spectrum obtained with a method named "hot electron capture dissociation" (HECD) [18]. In HECD electrons with an energy of around 10 eV first excite the molecule, then thermalise before they are captured as low energy electrons. In HECD spectra some of the most abundant fragments are due to secondary fragmentation caused by the large electron energy which is transferred to the molecule. In the HECD SRP spectrum a peak corresponding to a z_4 fragment that has lost a side chain group from leucine and become a so called w_4 ion is a prominent peak. In the present Na spectrum this peak is missing which indicates that high energy electron capture-induced fragmentation in an alkali metal target is closer to ECD than to HECD. This observation is in good accordance with a simple picture of the capture process where a low energy quasi-free electron is transferred from a loosely bound state on the Na atom to the dication.

In order to investigate the role of the ionization potential of the target atom we have performed fragmentation studies of Substance P and SRP also in a Mg target. The ionization potential of Mg is 7.64 eV while it is 5.14 eV for Na [17]. In Figs. 4 and 5 we compare the relative intensity of fragment ions normalized to the intensity of $[M + 2H]^+$ ions for both Substance P and SRP in Na and Mg. It should be

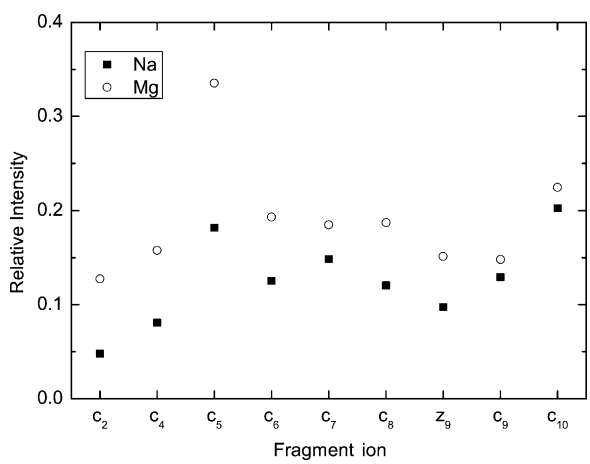

Fig. 4. Relative intensity of fragment ions of Substance P with Na and Mg target. The intensities were normalized with electron capture intensity.

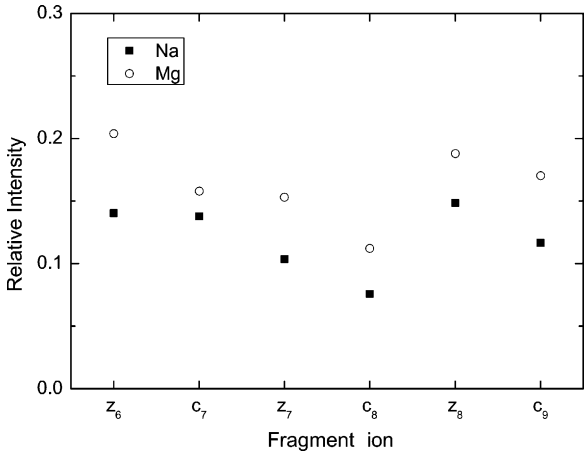

Fig. 5. Relative intensity of fragment ions of SRP with Na and Mg. The intensities are normalized with electron capture intensity.

noted that the relative peak heights are similar in the two target gases. The correlation factor between the relative intensities of c and z fragments is 0.86 for both precursor ions. Thus, the binding energy of the active target electron does not seem to play a major role as long as electron capture is the dominating reaction channel in the collision. This observation supports the idea about a non-ergodic character of the electron capture-induced fragmentation process.

4. Conclusions

It has been demonstrated that high energy electron capture from atomic targets with low ionization potential is an efficient fragmentation process in tandem mass spectrometry. Like in ECD where low energy-free electrons are captured by cations we observe cleavage of most backbone amide bonds. It is argued that the Na spectrum of Substance P can be considered as a sum spectrum of a high energy CID part and an ECD part. The high energy CID part can be obtained separately by using Ne as a target and the difference spectrum is almost identical with a ECD spectrum. The present fragmentation method is thus complementary to ECD and can be used in beam experiments where low energy electron targets are difficult to implement.

Acknowledgements

This work has been supported by the Danish National Research Foundation through the Aarhus Center for Atomic Physics (ACAP). We thank Roman Zubarev and Frank Kjeldsen for assistance and helpful discussions.

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