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Mechanism of Formation of Alkali Metal Cation Adducts in Plasma Desorption Mass Spectrometry of Biomolecules

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Experimental data on correlations between the directions of the incident primary ion and the ejected protonated $[M + H]^+$ and alkali metal adduct $[M + Cs]^+$ molecules for three peptide samples with an incident beam of 72.3 MeV $^{127}I^{14+}$ ions are reported. Measurements were carried out in a linear time-of-flight mass spectrometer by monitoring the secondary ion yield as a function of electrostatic deflection in a direction perpendicular to the spectrometer axis. No difference was observed in the direction in which $[M + H]^+$ and $[M + Cs]^+$ ionic species are preferentially desorbed. The results obtained suggest that alkali metal cation attachment to biomolecules in plasma desorption mass spectrometry is realized in a close spatial location and time interval with protonation. Formation of ion-molecule complexes occurs at an early stage of the desorption event and precedes their ejection into the gas phase.

INTRODUCTION

In plasma desorption (PD) mass spectrometry (MS),¹ fast primary ions (with energies in the range of 1 MeV per nucleon) are used for the generation of gas-phase ions of biomolecules with relative molecular masses of several thousands. PDMS was the first of several desorption ionization techniques [organic secondary ion mass spectrometry (SIMS), fast atom bombardment (FAB), laser desorption (LD)] that allowed mass spectral studies of non-volatile and thermally labile bio-organic compounds. While different aspects of the sputtering process involving fast primary projectiles have been investigated and thoroughly reviewed,²⁻⁷ there is less information regarding secondary ion formation. Several ionization mechanisms for PD have been discussed.⁴⁻⁶ Recently it has been shown⁸ and confirmed experimentally^{9,10} and theoretically¹¹⁻¹³ that a pressure pulse (shock wave) induced by the primary ion ultimately leads to the desorption of large intact biomolecules. The main features of the pressure pulse picture^{11,12} include the creation of large energy gradients in a cylinder (ion track), axially symmetric to the direction of the incoming ion. The energy gradient results in propagation of a pressure pulse in an outward direction, radially to the ion track. Thus a non-diffusive momentum transfer to a volume of sample biomolecules causes their ejection. The directional correlation effect⁸ is a consequence of the direct momentum transfer from the primary ion track to the large biomolecules desorbed. That effect is observed as an ejection of higher molecular mass compounds preferentially at an angle off the direction of the incoming ion. This is in contrast to lower mass secondary ions, which have ejection

angle distributions symmetric along the normal to the surface.⁹ Molecular dynamics simulations have also been employed and the directional correlation effect has been 'confirmed' in a computer modelling experiment.¹³

The correlation between the directions of bombarding ions and sputtered particles may be used as a probe, allowing for a better insight into both the spatial and temporal sequence of the ionization/desorption events in PD in general.⁹ It has also been established that the ratio of positively charged to neutral molecules ejected after heavy-ion impact in PD is of the order of 10^{-3} for the case of luteinizing hormone-releasing hormone (LHRH).¹⁴ Enhancement of the secondary ion yield is thus obviously desirable, particularly for lowering the detection limits in PDMS. Therefore, studies of ionization mechanisms contribute not only to the understanding of the PD phenomenon itself but are also of practical interest.

In this study we tried to elucidate the alkali metal cation attachment (cationization) mechanisms in PD. We attempted to resolve a major issue, namely whether cationization precedes desorption or whether it is a gas-phase type of process with ions and neutral species recombining in the selvedge.^{4,5,15} To this end we compared the directional correlation between the incident primary particles and the ejected $[M + H]^+$ and $[M + Cs]^+$ molecular ions. Data on directional correlation effects for pseudomolecular protonated and alkali metal adduct ions were taken in a linear time-of-flight (TOF) mass spectrometer by monitoring secondary ion yield as a function of voltage, applied to a pair of deflection plates. The secondary ions were electrostatically deflected in a direction perpendicular to the spectrometer axis. Three peptides in the RMM range 500-2000 were studied: leu-enkephalin (RMM 555), LHRH (RMM 1181) and renin substrate (RS) (RMM 1801).

EXPERIMENTAL

Commercially available peptide samples (Sigma Chemical) were used without further purification. Samples were prepared according to known procedures [16]. Droplets of the peptide (4 μl , dissolved in water + 0.1% trifluoroacetic acid, 10 $\mu\text{g } \mu\text{l}^{-1}$) and caesium iodide (2 μl , dissolved in water-ethanol (3:7), 10 $\mu\text{g } \mu\text{l}^{-1}$) solutions were co-deposited over the electro-sprayed nitrocellulose film on a silicon backing and were allowed to dry. Of course, the water rinsing step in order to remove inorganic salt impurities was omitted in the sample preparation procedure. A caesium salt was chosen for different reasons: first to allow the distinct separation of the $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{Cs}]^+$ ion peaks, and second to have a cation and a peptide molecule with comparable masses for kinematic reasons.

The experimental set-up for the studies of the directional correlation between incident particles and secondary ions has been described in detail elsewhere.^{8,9} Primary 72.3 MeV $^{127}\text{I}^{14+}$ ions from the Uppsala EN-tandem accelerator bombarded the target from the front at 45° incidence angle. Two sets of metal plates were used to deflect the initially accelerated desorbed secondary ions in x and y directions (see inset in Fig. 1). The plates, separated by a distance of 0.8 cm, were placed 5.0 cm along the axis (z -direction) of a linear time-of-flight mass spectrometer. The ion accelerating voltage was +12 kV. The yield of the positive secondary ions as a function of the voltage applied to the x -deflection plates was monitored using a standard PD mass spectrometric TOF data acquisition system.⁹ The x -deflection plates deflect the ions in a plane formed by the line of incoming primary ions and the TOF-spectrometer axis. The voltage on the set of y -deflection plates is adjusted to maximize the ion yield in order to compensate for an eventual non-parallel arrangement between the target surface and the spectrometer entrance grid, and is kept constant during the experiment. Between 2×10^5 (for leu-enkephalin and LHRH) and 4×10^5 (for RS) start count events were accumulated for each measurement point with an intensity of the primary beam between 1000 and 2000 ions s^{-1} . The secondary ion yield distributions were converted to radial velocity distributions using the procedure described previously.^{8,9}

RESULTS AND DISCUSSION

In all instances we observed abundant $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{Cs}]^+$ ion peaks with almost similar intensity. In the case of leu-enkephalin we also observed an intense peak corresponding to the $[\text{M} + 2\text{Cs} - \text{H}]^+$ pseudo-molecular ion. It is now well established that higher mass compounds (data for $[\text{M} + \text{H}]^+$) are ejected preferentially at an angle off the normal to the surface, in contrast to lower mass fragment ions.⁸⁻¹⁰ This directional correlation effect can be illustrated for the case of $[\text{M} + \text{H}]^+$ and CH_3^+ ions from RS (Fig. 1). There is a difference between the centroids of the distributions for CH_3^+ ions on one side and $[\text{M} + \text{H}]^+$ and other secondary ions with a higher mass on the other side. The

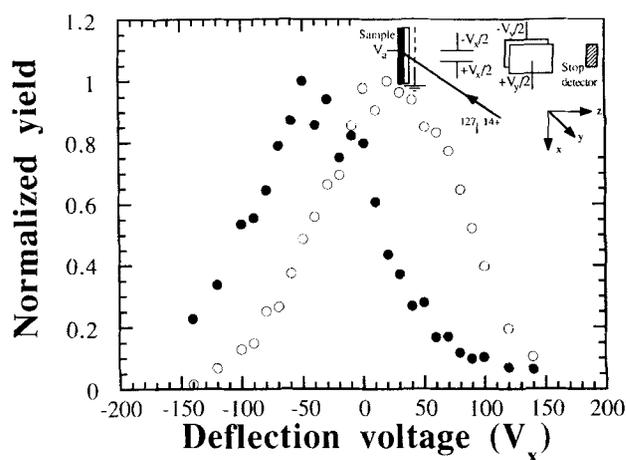


Figure 1. Ion yield distribution for (○) CH_3^+ and (●) $[\text{M} + \text{H}]^+$ ions for renin substrate as a function of the deflection voltage. The inset shows a schematic diagram of the experimental set-up

difference increases with increasing mass of the compounds studied:⁹ 50 V for leu-enkephalin, 75 V for LHRH and 100 V for RS. On the other hand, the Cs^+ cation distribution as a function of deflection voltage almost completely overlaps that for CH_3^+ ion in all three cases (Fig. 2). The distributions for the lower mass ions are symmetric and peak along the normal to the surface, within the limits of the experimental error, as has been reported previously.⁹ Following the procedure outlined previously,^{8,9} one can estimate the radial velocity distribution of the ejected Cs^+ and also $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{Cs}]^+$ ions. The zero offset of the radial velocity distribution scale corresponds to the centroid of the distribution for lighter ions, e.g. CH_3^+ . It should be noted that the Cs^+ ions desorbed from all three samples have the same radial velocity distribution (Fig. 3). The main experimental results of this study are illustrated in Fig. 4, showing the radial velocity distributions for the $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{Cs}]^+$ ions for the cases of RS, LHRH and leu-enkephalin. Within the limits of the experimental error there is a close overlap of the radial velocity distributions for both $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{Cs}]^+$ for all three peptides examined. One

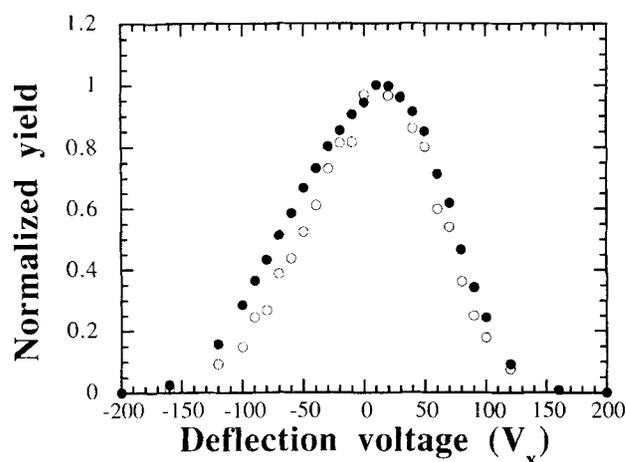


Figure 2. Yield distribution for (○) CH_3^+ and (●) Cs^+ ions as a function of deflection voltage for a leu-enkephalin sample.

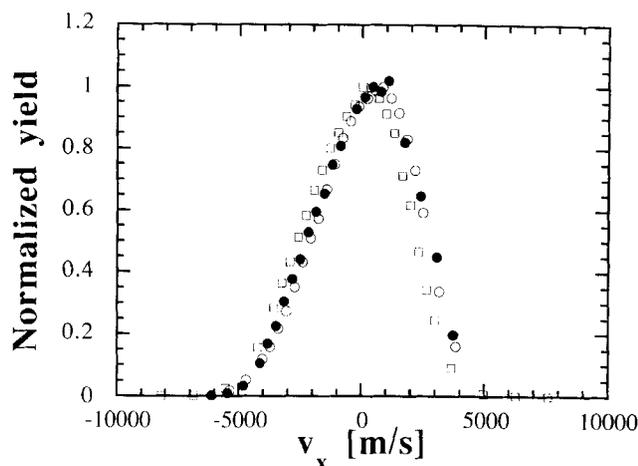


Figure 3. Cs^+ cation radial velocity distribution from the three samples: (\square) leu-enkephalin; (\bullet) LHRH; (\circ) RS.

should also note the finding that the $[\text{M} + 2\text{Cs} - \text{H}]^+$ pseudomolecular ion of leu-enkephalin has the same radial velocity distribution as the protonated and singly cationized ions (Fig. 4(c)). As axial velocity distributions for higher mass ions have been found to be similar,¹⁷ we can infer that $[\text{M} + \text{H}]^+$, $[\text{M} + \text{Cs}]^+$ and $[\text{M} + 2\text{Cs} - \text{H}]^+$ ion species for the studied peptides are on average desorbed in the same spatial direction.

Formation of adducts of the neutral sample molecule (M) with alkali metal cations (Alk^+) is a major ionization process in PD. The presence of Na^+ and K^+ peaks (probably from trace impurities) is an inherent feature of all PD mass spectra obtained from bioorganic samples electrosprayed on the target.^{2,4} In many instances multiple H^+/Alk^+ exchange in the molecule leads to species of the type $[\text{M} + n\text{Alk} - (n-1)\text{H}]^+$, depending on the number of acidic groups in the molecule. Cationization, resulting in (the so-called 'pseudomolecular') even-electron ions, is one of the common features of PD and the other desorption-ionization techniques, including SIMS, FAB and LD. Its potential in studies of polar biomolecules was realized early in the analytical applications developed for, e.g., field desorption¹⁸ and LD.^{19,20} This is why recipes for sample preparation in several techniques often include addition of alkali metal salts.^{19,20} On the other hand, it has been recognized that the presence of different monovalent or divalent cations in samples submitted to PDMS lowers the molecular ion abundance significantly and broadens the observed peaks.¹⁶ The results obtained with the introduction of the nitrocellulose sample preparation technique in PDMS¹⁶ are attributed in part to the ability for removal of all alkali metal cations from the sample surface. We also note that studies of cationization in ^{252}Cf PDMS have been reported,^{21–23} mainly for determining the binding properties of different classes of compounds to alkali metal cations. Phospholipid (dipalmitoylphosphatidylcholine)-alkali metal cation (Na^+ , K^+ , Rb^+ and Cs^+) adduct formation has been examined.²¹ Knysh *et al.*²² discussed the ion-molecule complex formation for two cyclic peptide antibiotics, enniatin B and valinomycin, with Na^+ , K^+ and Cs^+ . Malhotra *et al.*²³ measured the

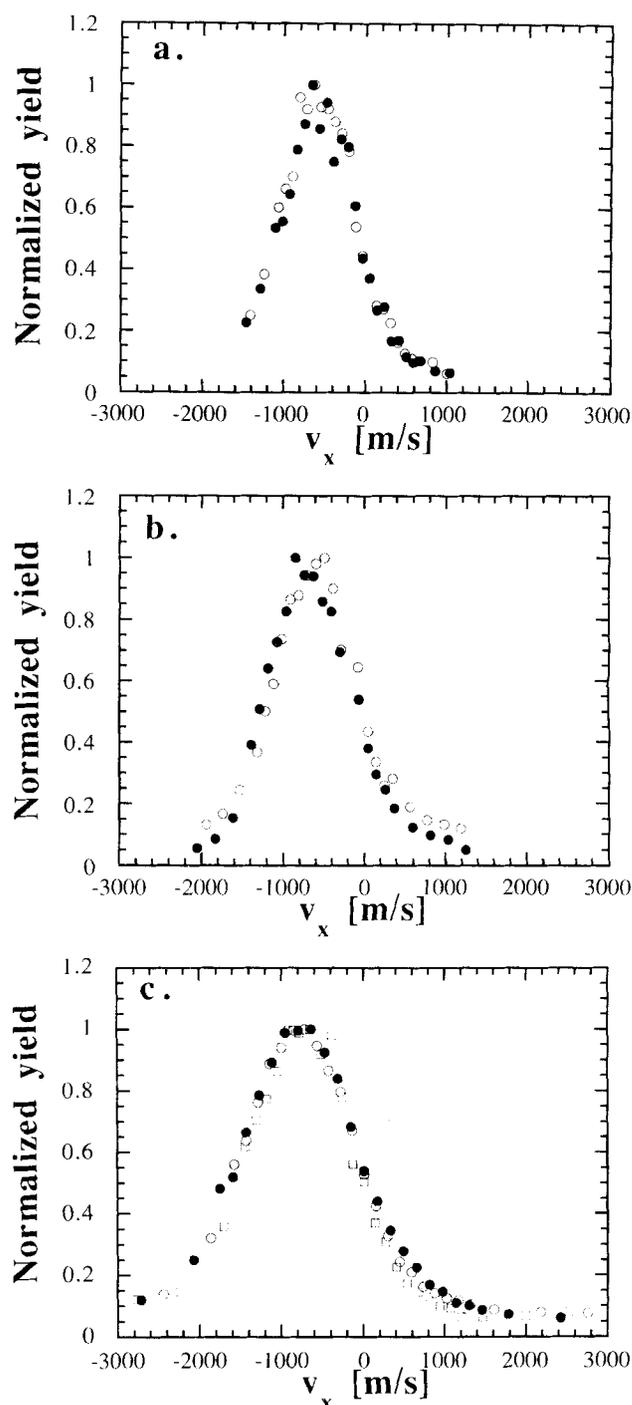


Figure 4. Radial velocity distributions for the PD quasimolecular ions from (a) RS, (b) LHRH and (c) leu-enkephalin. (\bullet) $[\text{M} + \text{H}]^+$; (\circ) $[\text{M} + \text{Cs}]^+$; (\square) $[\text{M} + 2\text{Cs} - \text{H}]^+$.

relative binding ratios to Li^+ , Na^+ and K^+ of seven crown ethers. A corollary of these studies, pertinent to our discussion, is that the ratio of pseudomolecular ion abundances reflects qualitatively the alkali metal cation binding affinity to the studied compounds in solution. The observed correlation between gas-phase ion abundances and solution alkali metal cation binding affinity may be an indication of already performed ion-molecule complexes in the condensed phase, from which they are desorbed after the particle impact.

Similarities in the mass spectra of organic molecules obtained by different techniques such as FAB, SIMS and LD have prompted a unified description of the ionization–desorption even at least in its later stages.¹⁵ It has been proposed that many of the ionization processes (including cationization) during the desorption of organic molecules by FAB, SIMS and LD take place in the selvedge region.¹⁵ Delayed emission experiments in LD confirm that cation attachment to polar molecules takes place in the gas phase.^{19,20} Although this is true for lower laser powers (10^6 W cm^{-2}), at higher energy densities ($10^{10} \text{ W cm}^{-2}$) direct emission of cationized species from the surface is favoured²⁴ owing to the too short-lived selvedge region and/or higher temperature of the generated plasma. There have been plausible suggestions for the sequence of the different events resulting in the formation and emission of the charged biomolecules in PDMS. Macfarlane *et al.*⁴ proposed that whereas the gas-phase dissociation of molecular aggregates can lead to the formation of odd-electron positive and negative molecular ions, cationization and proton-transfer reactions between interstitial species and the neutral biomolecules are initiated in the condensed phase. These aggregates, possessing a net preformed ionic character, are then ionized during the desorption in a surface ionization type process.

More recently, Macfarlane *et al.*⁶ have elaborated on the process, advancing a mechanism that involves solid-state formation of ion pairs and their subsequent dissociation after ejection via different charge-competing paths, resulting in both neutral and charged (protonated and cationized) species. On the other hand, extension of the selvedge region concept from LD and SIMS to PD has prompted the suggestion of eventual gas-phase alkali metal cation attachment after the desorption of neutral molecules and cations^{5,15} in PD. We are able to rule out that concept for gas-phase alkali metal cation attachment based on the results we have obtained. Eventual post-desorption gas-phase collisions and ion–molecule association reactions (no matter how unlikely they are, given the low secondary ion yield per incoming primary ion in PD) would result in smearing and loss of the directional correlation of the resulting pseudomolecular ion adducts. The momentum conservation in a post-desorption gas-phase recombination of, e.g., Cs^+ ion (whose angular distribution peaks at 0°) and a leu-enkephalin molecule (with a mass around 4 Cs masses (would in any case lead to a pronounced shift in the radial velocity distribution between $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{Cs}]^+$, which is not observed experimentally. Such a kinematic effect should be even more enhanced in the case of consecutive gas-phase recombination reactions leading to $[\text{M} + 2\text{Cs} - \text{H}]^+$ pseudomolecular ion of leu-enkephalin. The experimental result is the opposite, i.e. the radial velocity distributions for the three different pseudomolecular ions closely overlap (Fig. 4(c)). The theoretical possibility of the reverse process, sputtering of clusters, followed by gas-phase uncluster decomposition of the type $[\text{M} + (n + 1)\text{Cs} - (n - 1)\text{H}]^+ \rightarrow [\text{M} + n\text{Cs} - (n - 1)\text{H}]^+ + \text{Cs}$, where $n = 0, 1, 2, \dots$, is also ruled out on the basis of this observation. Eventual gas-phase decomposition would cause broadening of the radial kinetic energy distribution for the pseudomolecular

$[\text{M} + \text{H}]^+$ and $[\text{M} + \text{Cs}]^+$ ions owing to translational energy release (of the order of 0.1 eV) in that process. This would lead to a noticeable offset of the respective radial velocity distributions, which is not the case (Fig. 4(c)). Analytical models and molecular dynamic simulations,^{9,11–13} based on the shock-wave concept, predict higher radial velocities for ions ejected closer to the ion track. The results obtained by us suggest that within the limits of the experimental error both $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{Cs}]^+$ ions are ejected with the same radial velocity, i.e. at a similar distance from the ion track core. The same is also valid for the $[\text{M} + 2\text{Cs} - \text{H}]^+$ pseudomolecular ion of leu-enkephalin.

The experimental results presented here do not allow one to distinguish between preformed cation complexes, deposited on the target, and cationization reactions at an early stage before desorption into the gas phase. It is unlikely that solution ion–molecule complexes, existing in the liquid phase from which they are deposited to the solid target, could survive intact the transfer step (especially for the case of the harsher electrospray deposition technique). It is certain, though, that the formation of alkali metal cation–biomolecule adducts in PD precedes their ejection into the gas phase by a pressure pulse (shock wave) type of mechanism. A rough estimate of the time scales involved can be obtained from the molecular dynamics simulations of the sputtering process for larger biomolecules:¹³ the first molecules leave the surface at $\sim 10^{-11}$ s, whereas the ejection yield saturates at $\sim 10^{-10}$ s. A scenario involving the synchronous movement through the condensed phase (caused by the pressure pulse) of a neutral sample molecule and one or more alkali metal–halide ion pairs, and their recombination and ionization (by, e.g., surface ionization⁴) at a later time seems much less likely. First the ion pair(s) and the neutral molecule(s) should be in close spatial proximity. The stability of such a neutral molecule–ion pair complex is very low in order to preserve the relative specific orientation facilitating, e.g. the intermolecular hydrogen transfer and substitution reactions in the case of pseudomolecular ion formation. Experiments reporting²⁵ the observation of gas-phase alkali metal cation–polar molecule complexes from a sample layer covering a layer of caesium iodide have been interpreted in terms of cation diffusion along the ion track. Therefore, we suggest that the cationization reaction, resulting in preformed ion–molecule complexes, may be eventually triggered by the pressure pulse. The ultimate result of the propagating wave will then be ejection of these complexes into the gas phase at an angle off the direction of the incoming beam.

CONCLUSION

The radial velocity distribution of secondary ions ejected in a plasma desorption event carries an important message for the ion formation mechanism. We have found that both $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{Cs}]^+$ ionic species for peptides with different masses are preferentially desorbed in a direction roughly perpendicular to the ion track, thus preserving a ‘memory’ for the

direction of the incident ion. These results suggest that both alkali metal cation attachment and protonation in PDMS are realized in a close spatial location and time interval within a similar distance from the ion track core. Although it is not possible to distinguish between preformed ions, deposited on the target, and cationization reactions at an early stage of the PD event, it is certain that the formation of alkali metal cation-biomolecule complexes precedes their ejection into the

gas phase by a pressure pulse (shock wave) type of mechanism.

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