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On molecular ion formation by hydride abstraction in plasma desorption mass spectrometry

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Abstract

Positive molecular ion formation in plasma desorption (PD) by hydride abstraction leading to $[M - H]^+$ molecular ions is not a frequently observed phenomenon An example of a compound that exhibits an $[M - H]^+$ molecular ion in its PD spectrum is the neutral lipid cholesterol Data on the initial radial velocity distribution of the $[M - H]^+$ ion of cholesterol are presented and compared to data on the radial velocity distribution of the M^+ molecular ion of a structurally similar compound (ergosterol) The distribution for $[M - H]^+$ cholesterol ions is broader and its centroid is closer to zero radial velocity than the corresponding distribution for ergosterol M^+ ions These data support a model suggesting formation of $[M - H]^+$ by a "self" chemical ionization type reaction

Keywords plasma desorption, hydride abstraction, cholesterol, radial velocity distribution, ion formation

Introduction

Plasma desorption (PD) mass spectrometry has been one of the first particle bombardment techniques successfully applied for formation of gas-phase ions of non-volatile and thermally labile biomolecules [1] PD has rapidly evolved [2,3] and has been employed mainly for studies of peptides in the mass range up to 45 kDa [2–6] The potential of the technique for studies of other classes of compounds has also been illustrated [2,3,6] The usefulness of PD for assignment of molecular weights is determined by the fact that various types of intact molecular ions from different species are formed and observed in the mass spectra of the respective compounds As in other desorption ionization techniques [5] the main type of positive (quasi)-molecular ions are those corresponding to the neutral sample molecule with attached proton or (most often) alkali metal cation $[M + (n + 1)Alk - nH]^+$, where $n = -1, 0, 1, 2$ Radical (odd-electron) molecular ions M^+ obtained by loss of an electron from the neutral molecule have also been observed in PD mass spectra of biomolecules Examples of such compounds are ergosterol and chlorophyll (the latter exhibits both even-electron (protonated) and radical molecular ions) We have recently studied specific aspects of the formation mechanism of cationized adducts and radical ions in PD by using as a probe the initial radial velocity distributions of the ejected ions [7–9]

So far there have been only a few cases when

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$[M - H]^+$ molecular ions are encountered in PD mass spectra of organic molecules Pannell et al have reported the formation of $[M - H]^+$ molecular ions in PD of the neutral dyes "malachite green base" and "aluminon" [10] They suggested that the stability of the triarylcarbonium ion formed by hydride abstraction is the driving force leading to the $[M - H]^+$ ion PD mass spectra of volatile hydrocarbons such as *n*-decane (from targets cooled to liquid N_2 temperature) also exhibit intense $[M - H]^+$ molecular ions [11] Another specific example of a biomolecule exhibiting an $[M - H]^+$ ion in PD is the neutral lipid cholesterol In this study the initial radial velocity distribution of ejected cholesterol molecular ions is reported and used for elucidating the mechanism of molecular ion formation by hydride abstraction in PD It is also compared to previously obtained data on the initial radial velocity distribution of radical and protonated positive molecular ions from various biomolecules [7–9], including the structurally similar compound ergosterol We argue that the $[M - H]^+$ even-electron ion in PD is formed in a "self" chemical ionization type reaction which mimics the well-known gas-phase ion molecule deprotonation reactions of low proton affinity molecules such as saturated hydrocarbons [12,13]

Experimental

PD mass spectra were obtained on a single-stage reflectron time-of-flight (TOF) mass spectrometer (Fig 1) described in detail elsewhere [14] The spectrometer was installed on one of the beam-lines of the Uppsala EN tandem accelerator which generated 72 3 MeV $^{13+}$I ions for bombardment of the sample from the front at 45° angle of incidence (Fig 1) The procedure for measurement of the initial radial velocity distribution involved monitoring the secondary ion yield as a function of electrostatic deflection in an *x* direction perpendicular to the target surface normal (see Fig 1 for coordinate system assignment) Conversion from deflection voltage units to radial velocity units was performed in a straightforward manner using the

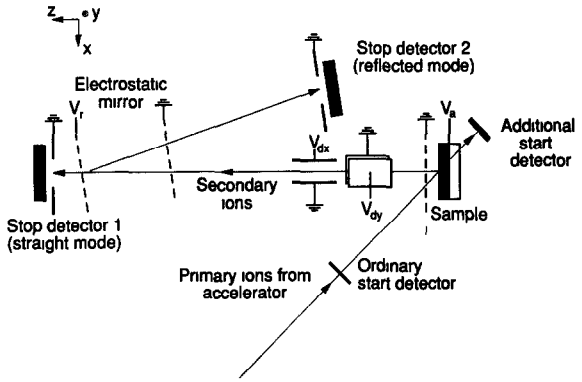
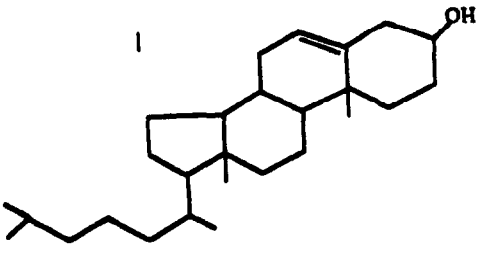


Fig 1 Schematic diagram of the experimental set-up including the reflectron time-of-flight mass spectrometer

geometric parameters of the TOF instrument [8,9,14] The term radial velocity is used here for brevity, although only the velocity in the *x* direction is monitored, the reason being that the initial velocity distribution along the *y* direction is symmetric [8,9,14,15] Standard PD TOF electronics including a multistop time-to-digital converter with a 0 5 ns resolution per channel was used for data collection [3,14] The number of start events, accumulated for each deflection voltage value was $2\,5 \times 10^5$ The vacuum in the system, maintained by two cryopumps, was better than 5×10^{-9} Torr

Cholesterol ($C_{27}H_{46}O$, monoisotopic mass 386 321 u, **I**) and ergosterol ($C_{28}H_{44}O$, monoisotopic mass 396 307, **II**), were purchased from Sigma Chemical Co (St Louis, MO, USA) and were used without further purification The samples were prepared by dissolving in acetone and spin-coating the solution on Si targets



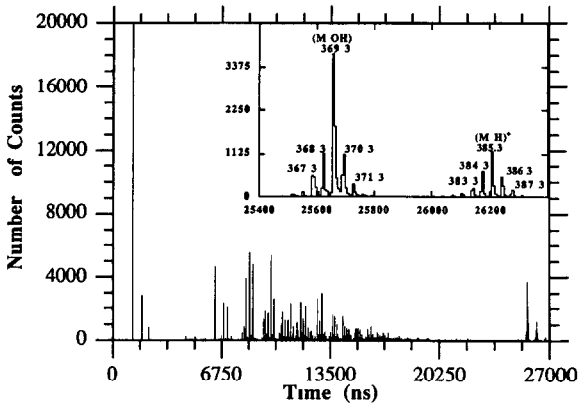


Fig 2 Positive ion PD mass spectrum of cholesterol

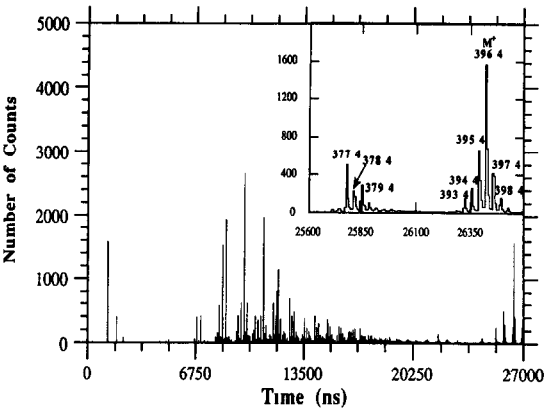
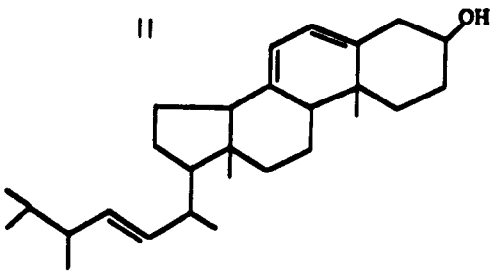


Fig 3 Positive ion PD mass spectrum of ergosterol



Results and discussion

The positive ion PD mass spectrum of cholesterol, taken in the reflected mode with base line (unit) resolution in that mass range, is shown in Fig 2 The molecular ion region (inset of Fig 2) contains the $[M - H]^+$ (quasi)molecular ion peak as a main peak at m/z 385 The measured ratio of intensities of peaks at m/z 385 and 386 is 1 0 56, which suggests that the degree of H elimination from M^+ is rather high (one also has to consider the ^{13}C isotope contribution, attributing with 27% of the relative intensity of the peak at, for example, m/z 385 to the intensity of the peak at m/z 386) Peaks at m/z 384, 383 and 382 (0 57, 0 22 and 0 08 respective intensity ratios relative to m/z 385 peak) suggest that the process of dehydrogenation is readily extended with more than one H atom being eliminated during molecular ion formation There is only one major fragment ion at m/z 369, which is also the most intense peak in the higher mass region of the spectrum (above m/z 60) It corresponds to a loss of OH radical from the M^+ ion at m/z 386 (by analogy with chemical ionization, [12] loss of H_2O from $[M + H]^+$ leading to the m/z 369 ion cannot be ruled out either) The yield of $[M - H]^+$ ions (number of counts in the peak divided by the total number of start counts) is $2\ 57 \times 10^{-3}$ Molecular cluster ions are observed in the positive ion mode with masses corresponding to $[nM - H]^+$ (n = 2–4) In the negative ion spectrum (not shown) a peak corresponding to $[M - H]^-$ as well as peaks corresponding to cluster ions of the type $[nM - H]^-$ (n = 2,3,4) are encountered

The positive ion PD mass spectrum of ergosterol is presented in Fig 3 Unlike cholesterol, it exhibits M^+ as the most intense peak in its spectrum at m/z 396 with an ion yield of $8\ 43 \times 10^{-3}$ Dehydrogenation is also observed the intensity ratios for peaks at m/z 395, 394 and 393 relative to M^+ are correspondingly 0 39, 0 13 and 0 07 The loss of an OH radical from the molecular ion (or H_2O elimination from an $[M - H]^+$ precursor ion), leading to an ion at m/z 379, is less pronounced

The yield of a low mass ion CH_3^+ and of $[M - H]^+$ for cholesterol as a function of the x deflection voltage is given in Fig 4 It is evident that both distributions are closely matching That corresponds to lower mean radial velocity for cholesterol ions (see below) because the mean radial velocity for low mass fragments (including CH_3^+) is zero [8,9,16] The corresponding initial radial

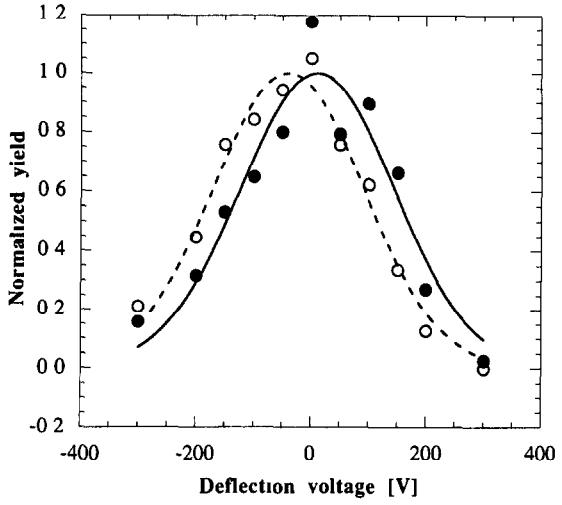


Fig 4 Secondary ion yield distribution in the reflected mode as a function of x deflection voltage for $[M - H]^+$ (●) and lower mass fragment, e g CH_3^+ (○), for cholesterol The data points have been fitted with a gaussian curve

velocity distributions for the molecular ion and for the major fragment of cholesterol are given in Fig 5(a) The velocity distributions of $[M - H]^+$ ion for cholesterol and M^+ for ergosterol are compared in Fig. 5(b) Ergosterol molecular ions have almost two times higher mean radial velocity compared to cholesterol molecular ions ($-562 \pm 21\,m\,s^{-1}$ versus $-291 \pm 62\,m\,s^{-1}$) The width of the distribution at half maximum for the cholesterol ions is also 1 3 times larger than that for ergosterol ions

Molecular ion formation by hydride ion transfer leading to even-electron ions according to the reaction $X^+ + M \rightarrow XH + [M - H]^+$ has been described and discussed for different ionization techniques, including chemical ionization [12,13] and fast atom bombardment FAB [17] Baldwin et al [17] have demonstrated that hydride abstraction is characteristic of FAB mass spectra of compounds containing a long saturated hydrocarbon chain It is not affected by different matrices and is observed even when pure liquid compounds are examined by FAB These authors argue that hydride abstraction is not associated with heteroatoms and/or polar groups present in these compounds, and it may also take place in the gas

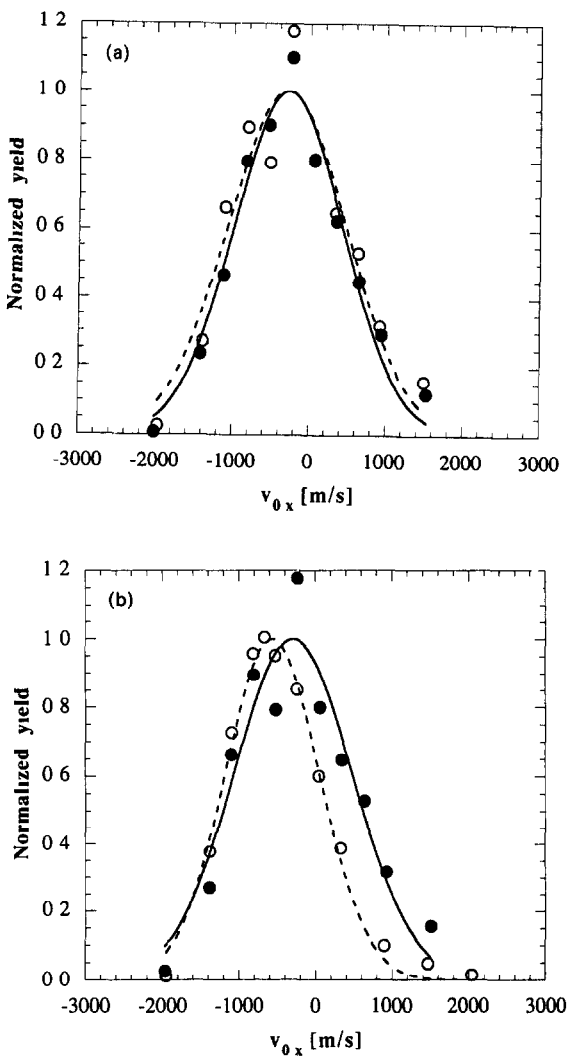


Fig 5 (a) Radial velocity distribution of the cholesterol $[M - H]^+$ (●) and $[M - OH]^+$ (○) ions (zero on the velocity axis corresponds to the centroid of the distribution of low mass ions (CH_{3+})) The data points have been fitted with a gaussian curve (b) Radial velocity distribution of the cholesterol $[M - H]^+$ (●) and ergosterol M^+ (○) molecular ions (data for ergosterol adapted from ref 8) The data points have been fitted with a gaussian curve

phase That argument may be extended to PD because hydride abstraction is more effective for more saturated compounds Cholesterol (I), for example, contains two double bonds less than ergosterol (II) and for these compounds even minor changes in the structure lead to different rates of hydride abstraction in PD Similar observations

have been made by Wien [11], who has obtained the PD spectra of various volatile compounds at subambient temperatures Both *n*-hexane and *n*-decane exhibit a strong degree of dehydrogenation (ions of the type $[M - nH]^+$) in contrast to aromatic compounds such as phenol, ethylbenzene and nitrobenzene [11] Similar behavior is characteristic of the well-known gas-phase ion/molecule reactions of low proton affinity molecules such as saturated hydrocarbons [12,13] Our interpretation of the formation of $[M - nH]^+$ ions in PD invokes the possibility of a gas-phase (selvedge [18]) "self" chemical ionization type reaction between desorbed species (including charged and neutral molecules and fragments) that leads to hydride abstraction Self-chemical ionization is a process typical of ion storage devices such as the ion cyclotron resonance mass spectrometer [19] and the ion trap detector [20], where the longer ion residence times (in the millisecond range) promote ion/molecule reactions even though the pressure is quite low Although the total particle yield in electronic sputtering per mega-electronvolt ion impact is rather low [4,5], an argument similar to the one forwarded by Pachuta and Cooks in a discussion of the selvedge concept [18] may justify the existence of pressure of the order of 10^4 Torr for more than 3×10^{-12} s during the PD ejection process We also note that the chemical ionization (methane as a reagent gas [12]) and FAB (nitrobenzylalcohol/thioglycerol matrix [21]) mass spectra of cholesterol contain both the $[M - H]^+$ and $[M - OH]^+$ peaks as major peaks

Further evidence for a "self" chemical ionization reaction leading to hydride abstraction comes from the comparison of the radial velocity distribution data of cholesterol and ergosterol ions (Fig 5) Various PD experiments have demonstrated the existence of a shift (termed directional correlation effect) between the initial radial velocity distributions of low mass (predominantly fragment) ions and higher mass molecular ions [9,15] We note that the low mass ions in PD are ejected with an angular distribution symmetric along the surface normal (zero mean radial velocity) as expected of a thermal evaporation process The asymmetry of the angular distribution of ejected higher mass ions in PD has been used as an illustration of the validity of the currently accepted pressure pulse (shock wave) model of electronic sputtering of biomolecules [4,5] We have also argued that the directional correlation effect bears implications for the ion formation process in PD [7,8,16] The initial radial velocity is a specific signature of the spatial location of ejected ions, as has been demonstrated for the case of cationized molecular ions and radical molecular ions of various biomolecules [7–9] They all originate from the outer region of the ion track formed by the incoming mega-electronvolt primary ion, unlike, for example, fullerenes that are formed in the hot plasma region of the infratrack and thus ejected with a velocity vector pointing in the direction of the primary ion [16]

The initial radial velocity data for cholesterol (Fig 5) demonstrate that the $[M - H]^+$ cholesterol molecular ion has an angular distribution which is closer to the surface normal In contrast ergosterol — a molecule with similar structure and molecular weight, exhibiting predominantly the radical molecular cation M^+— has angular distribution with a maximum in a direction further away from the normal, off the direction of the incoming mega-electronvolt primary ion Formation of cholesterol molecular ions in a selvedge process involving interactions between different ejected species results in directional randomization and "smearing" of the directional correlation, typical for ejection of biomolecular ions in PD We note that the "smearing" is due not only to purely kinematic effects It is well-known that the thermochemical condition for a hydride ion transfer reaction is its exothermicity, i e the hydride ion affinity of X^+ should be higher than the hydride ion affinity of the product $[M - H]^+$ [12] The kinetic energy release in that reaction may impart additional kinetic energy to the resulting $[M - H]^+$ ion and contribute to the experimentally observed broadening of the cholesterol $[M - H]^+$ ion radial velocity distribution as compared to ergosterol For that reason we also favor the proposed mechanism for

formation of the $[M - H]^+$ ion involving an ion/molecule reaction, rather than a unimolecular H_2 elimination from the $[M + H]^+$ precursor ion Thermochemistry (i e different hydride ion affinities) is most probably the reason for observation of a much higher degree of hydride ion abstraction for cholesterol than ergosterol

The fragmentation rate in a particular monomolecular reaction depends on the internal energy content of the corresponding precursor ion For instance, H_2O loss from cholesterol correlates with, for example, the ion source temperature in electron impact [22], chemical ionization [12] and hydrogen seeded supersonic molecular beam [23] mass spectrometry Water elimination in the surface-induced dissociation of cholesterol also scales with the incident kinetic energy [24] We infer by analogy that the higher rate of hydroxyl group loss from cholesterol compared to ergosterol in PD indicates higher internal energy content of desorbed molecular ions for the former compound In its turn this conclusion may serve as evidence for desorption of cholesterol ions from a locus with higher energy density closer to the ion track core That argument will also be an explanation for the lower mean radial velocity of cholesterol, because it has been demonstrated that the initial radial velocity correlates with the spatial location of ejected ions [9]

Conclusion

Formation of $[M - H]^+$ ions in PD mass spectrometry of biomolecules by hydride abstraction is not frequently encountered It is characteristic of compounds with lower proton affinity containing a saturated hydrocarbon function Practitioners in the field of PD mass spectrometry should be aware of that possibility which (although rare) might lead to complications in establishing molecular weights For example, predominant formation of $[M - H]^+$ in PD is characteristic of cholesterol We argue that the molecular ion in that case is formed by hydride abstraction in a "self" chemical ionization type reaction The $[M - H]^+$ cholesterol molecular ion has an angular distribution which is closer to the surface normal In contrast ergosterol — a molecule with similar structure and molecular weight, exhibiting predominantly the radical molecular cation M^+—has angular distribution shifted further away from the surface normal, off the direction of the incoming mega-electronvolt ion This observation supports the above suggestion for formation of cholesterol molecular ions in a selvedge process by ion/molecule reactions, thus resulting in smearing of the directional correlation, typical for ejection of biomolecular ions in PD

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