Sample Temperature Dependence of Plasma Desorption Mass Spectra of Biomolecules

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The effect of sample/substrate temperature on the ^{252}Cf plasma desorption (PD) ion yield of three different peptides with molecular weights between 231 and 5733 has been studied in the temperature range from −100 to +100 °C. The variation in the positive ion yield of the peptide molecular ions and lower mass fragment ions has been investigated as a function of the sample/substrate temperature. A decrease in the molecular ion yield has been observed at temperatures both higher and lower than ambient, whereas lower mass fragment ions show an increase in their yield at higher temperatures. Possible explanations of the above observations are discussed.

INTRODUCTION

In plasma desorption mass spectrometry (PDMS), introduced by Macfarlane and co-workers,[1] atomic ions with energy of the order of a megaelectronvolt per atomic mass unit from decaying ^{252}Cf nuclei are used to bombard surfaces of nonvolatile and thermally labile biomolecules. The velocity of these ions is higher than the Bohr velocity (0.22 cm/ns) and the ion–solid interactions for this velocity range are effected by excitation of the electronic subsystem of the solid.[2,3] PD is an example of the electronic sputtering phenomenon[3] which has been studied extensively during recent years.[3-5] The ability of PD to transfer intact large thermally labile biomolecules from the solid into the gas phase has been successfully exploited for mass spectral analysis of peptides with MW up to 40 000.[2,4,5] The applicability of PDMS for studies of protein–substrate and protein–protein interactions by employing the nitrocellulose adsorption technique[6] has also been illustrated.[7]

So far there have been only a few reports of PD mass spectra taken at temperatures differing from ambient temperature.[8-11] The effect of temperature on PD mass spectra of different substrates including two amino acids and inorganic dielectrics Eu_2O_3 and condensed H_2O has been studied by Matthäus and Moshammer.[8] Wien has investigated the PDMS of different volatile organic hydrocarbons by cooling the target to −180 °C.[9] The yield of both positive and negative molecular and low mass (including H, CN^-, and $C_3H_7^+$) ions from decane, nitrobenzene, phenol, and 1-methylnaphthalene has been monitored as a function of temperature in the range −180 to +25 °C.[9] Using the same setup, Wien has studied the PD mass spectra of frozen marine sediments.[10] We also note that the effect of heating upon the sputtering yield of isletlike gold films bombarded by fission fragments from ^{252}Cf was reported by Baranov et al.[11]

Up to now there have been no investigations on the effect of sample/substrate temperature upon the PD mass spectra of bioorganic molecules, larger than amino acids. In the present study we have attempted a more systematic investigation of this effect in the case of the PDMS of peptides. Three peptides were used—trialanine (tri-ALA, relative molecular mass (rmm) 231 Da), *N*-acetylrenin substrate (RS, rmm 1801 Da), and bovine insulin (INS, rmm 5733 Da). The variation of the molecular ion yield for these peptides in the temperature range from −100 to +100 °C was studied. The dependence of the ion yield of multiply-charged molecular and multimeric (cluster) ions on temperature has been monitored. The changes in intensity of peaks corresponding to lower mass fragment ions (e.g. immonium ions) as a function of the sample/substrate temperature has been also studied. Interpretation of the observed results in terms of protein stability as a function of temperature[12,13] has been suggested. The influence of the suspected residual gas condensation on the target upon cooling on the observed variations of molecular ion yield has been examined as well.

EXPERIMENTAL SECTION

Time-of-Flight Mass Spectrometer. The schematic diagram of the ^{252}Cf PD mass spectrometer which has been specifically designed for this project is shown in Figure 1. Unlike more widespread configurations of PDMS instruments[2] the sample block in this instrument was kept at ground potential in order to facilitate cooling and heating in the temperature range −100 to +100 °C. The sample was heated by a 4-W heating element while cooling was done initially through a cold finger immersed in a liquid N_2 filled dewar vessel. The heating rate was regulated using a variac and typical times for heating between +20 and +75 °C were around 20 min. Cooling of the sample was sped up by further modification which included circulation of liquid N_2 directly through the sample holder. Thus cooling of the sample from ambient temperature to −60 °C was effected in around 15 min. A liquid N_2 cooled Cu screen was mounted inside the vacuum chamber to minimize effects of residual gas condensation on the sample surface. The sample block and Cu screen temperatures were measured by thermocouples connected to TS 67 (Swema, Sweden) and 52J/K (J. Fluke) universal thermometers. A typical operating temperature for the screen was around −150 °C. A pressure of 10^{-7} Torr inside the chamber was maintained initially by means of an oil diffusion pump. The

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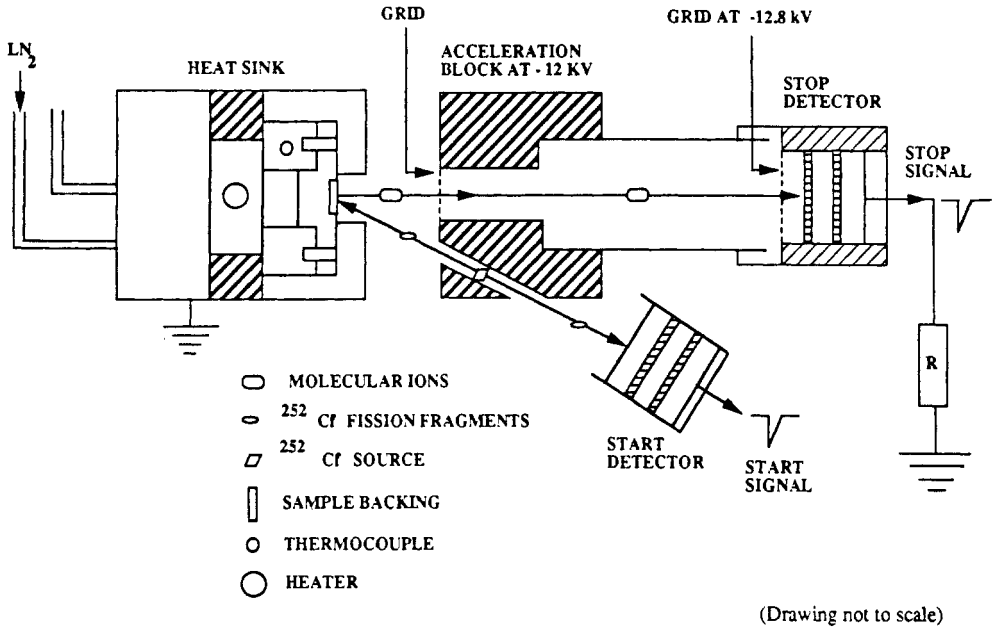

Figure 1. Schematic diagram of the time of flight mass spectrometer.

diffusion pump was later replaced by a 500 L/s turbopump in order to further improve the pressure to less than 10^{-7} Torr and have an oil-free vacuum in the chamber (a comparison of the operation of the spectrometer under different operating conditions is given below).

Fission fragments from a ^{252}Cf source were incident on the target surface from the front at around a 45° angle (Figure 1). A typical start count rate was around 500 counts/s. The positive ions ejected from the sample were accelerated toward an extraction grid in front of the flight tube, placed 5 mm away from the sample. Both the flight tube and the microchannel plate stop detector were floated at negative high voltage at different settings: −6.5 kV for tri-ALA and RS and −12 kV for INS. The stop detector anode was kept at ground. The signal from the stop detector was processed with standard PDMS electronics,[2] including a preamplifier (Ortec), a constant fraction discriminator (Ortec) and a multistop time-to-digital converter (Bioion Nordic, Sweden) with 1-ns time resolution. Final data collection and analysis were performed on an Atari STF 1040 computer.

Sample Preparation. All peptides were purchased from Sigma Chemical Co. (St. Louis, MO) and were used without further purification. Tri-ALA was dissolved in a mixture of trifluoroacetic acid (TFA) and acetic acid (1:5). The peptides RS and INS were dissolved in a mixture (1:1) of 0.1% TFA in water and ethanol at a concentration of 1 nmol/1 μL and adsorbed onto nitrocellulose, which was electrosprayed on stainless steel backings.[6] A standard procedure for preparation of the nitrocellulose-adsorbed samples was used.[6] Tri-ALA was directly electrosprayed on bare stainless steel backing since low-mass peptides do not adsorb onto nitrocellulose.

A new target design was introduced in order to minimize contributions from background ions desorbed from the area of the sample backings holder surrounding the sample itself. A stainless steel cylinder (10 mm in diameter) was placed coaxially with the ion optical axis of the spectrometer. Nitrocellulose was electrosprayed on the side of the cylinder facing the grid, and the peptides were adsorbed to its surface.

Between 2.5×10^4 and 10^5 start counts were collected in order to obtain a spectrum at a particular temperature. A reproducibility test was carried out with each sample in order to assess both the stability of the instrument and effects due to low secondary ion statistics. Several (typically six) mass spectra were collected for each sample at room temperature. The molecular ion yield fluctuation for the case of insulin was around 6%. At each temperature point more than one spectrum was collected and the data reported were averages of several runs. Relative molecular ion yields were normalized to the yield at room temperature. The heating and cooling were performed independently, each time starting with a fresh sample at room temperature. In order to estimate the effect of thermal treatment in vacuum on peptides the heated (respectively cooled) samples were allowed to relax to room temperature and ion yields before and after the thermal treatment were compared.

RESULTS AND DISCUSSION

The sample temperature dependence of the molecular ion yield for the different peptides studied is shown in Figures 2–4. The temperature dependence of low-mass fragment ions (immonium ions at m/z 70, 72, 86, 91, 110, and 120, originating from the amino acids constituting different peptides[14]), observed in the spectrum of INS, is presented in Figure 5.

These results demonstrate that the secondary molecular ion yield for all three peptides studied decreases both at higher temperatures and lower temperatures. Figures 2–4 indicate the existence of an optimal substrate temperature range for PD of peptides. The molecular ion yield for the different peptides studied changes with an increase of the sample temperature at different rates. The number of desorbed molecular ions (both singly and doubly charged) decreases upon heating more gradually for the larger peptides. In the cases of INS and RS, plots of relative molecular ion yield vs temperature show that the ion yield does not change appreciably in the temperature range −30 to +30 °C whereas in the case of tri-ALA the yield is stable until about +70 °C and then falls off rapidly. The yield decreases abruptly at around +70 °C both for the monomer ($[M + H]^+$ and $[M + Na]^+$) and the multimer ($[2M + H]^+$ and $[3M + H]^+$) molecular ions of tri-ALA (Figure 2). The trimer ion of tri-ALA (Figure 2d) exhibits a markedly different behavior from both the monomer (Figure 2a) and the dimer (Figure 2b) ions upon cooling. While the yields of the latter drop not very significantly upon cooling to around −100 °C, the yield of $[3M + H]^+$ increases almost 2 times from its ambient temperature value. That large increase is probably connected with higher stability of the desorbed cluster due to lowering of its internal energy (e.g. of the order of 0.01 eV per vibrational degree of freedom of the system upon cooling to −100 °C) and/or some other effects (see discussion below). It should be also noted that the $[M + Na]^+$ cationized adduct ion of tri-ALA is also more stable than the protonated monomer and dimer ions upon cooling (Figure 2c).

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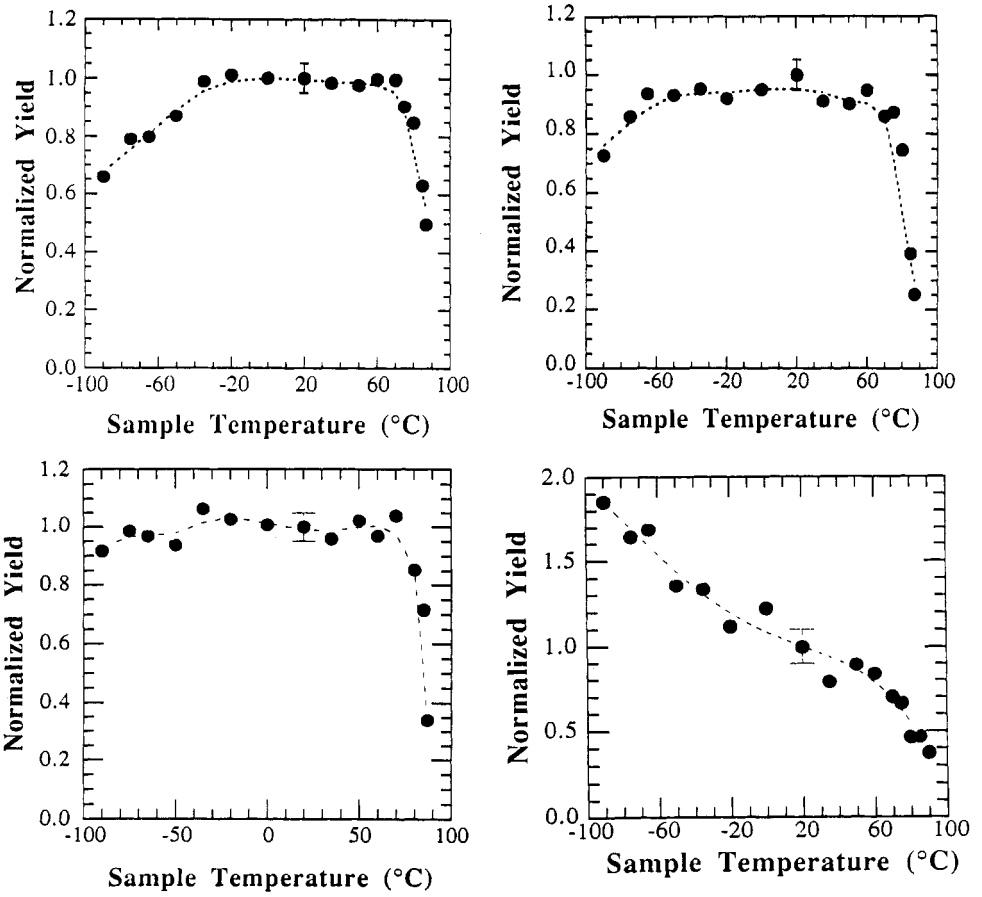

Figure 2. (a, Top left) relative yield of the $[M + H]^+$ ion of trialanine as a function of sample temperature. (b, Top right) relative yield of the $[2M + H]^+$ ion of trialanine as a function of sample temperature. (c, Bottom left) relative yield of the $[M + Na]^+$ ion of trialanine as a function of sample temperature. (d, Bottom right) relative yield of the $[3M + H]^+$ ion of trialanine as a function of sample temperature.

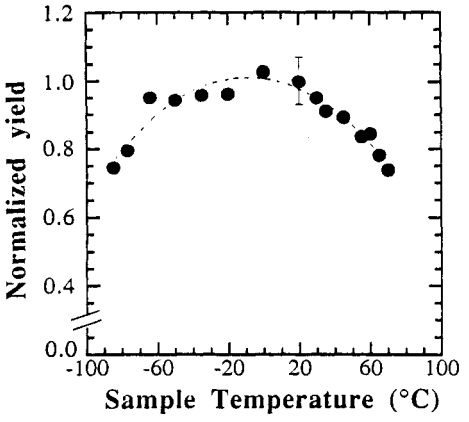

Figure 3. Relative yield of the $[M + H]^+$ ion of renin substrate as a function of sample temperature.

Our results confirm an earlier observation[8] on the effect of heating upon PD mass spectra of the amino acids valine and glycine. We demonstrate by comparing the molecular ion yield at room temperature before and after heating to +70 °C that heating leads to irreversible changes in the peptides. For instance the yield for $[M + H]^+$ molecular ions of tri-ALA drops 2.2 times, for $[M + H]^+$ of RS-1.4 times, and for $[M + H]^+$ of INS −1.5 times. We argue that the decrease in the ion yield upon heating is due to effects other than evaporation. Evaporation of these nonvolatile molecules, as suggested previously,[8] seems unfeasible even at the high-vacuum conditions present in the mass spectrometer chamber. While the abrupt drop upon heating for the small tripeptide may be attributed to thermal degradation of these relatively nonvolatile but thermally labile compounds, other factors may also contribute to the decrease in the molecular ion yield for the larger peptides.

The drop in the molecular ion yield of RS and INS both at higher and lower temperatures could be attributed to the fact that changes in temperature may cause a conformational change (thermal denaturation) of the peptide structure.[12,13] This may cause lower stability of the desorbed molecular ion in the gas phase due to the lower number of hydrogen and van der Waals bonds in an unfolded conformation. Thus increased fragmentation of the molecular ions will lead to overall lowering of the molecular ion yield. The validity of that argument at least upon heating is strengthened by the observation of increased yield of lower mass fragment (immonium) ions arising from bovine insulin (Figure 5). In parallel the increase in protein molecule–substrate (nitrocellulose) interactions due to unfolding and increase in the number of bonding sites would lead to increased surface binding energy and may cause lowering of the desorption yield as well. In order to substantiate the above interpretations we refer to the study on stability of peptide molecular ions in PDMS as a function of peptide secondary and tertiary structure.[15] In that study the decrease in molecular ion intensities and the increase in peak widths (enhanced molecular ion fragmentation) correlate with changes from folded to unfolded peptide conformation, induced by changes in the pH of the sample solution.

Other processes that may also contribute to the observed temperature effect include change in the charge state of the ejected particles with temperature and higher probablity for radiation damage of the unfolded proteins. Our findings may be also compared to recent studies on the effect of heating

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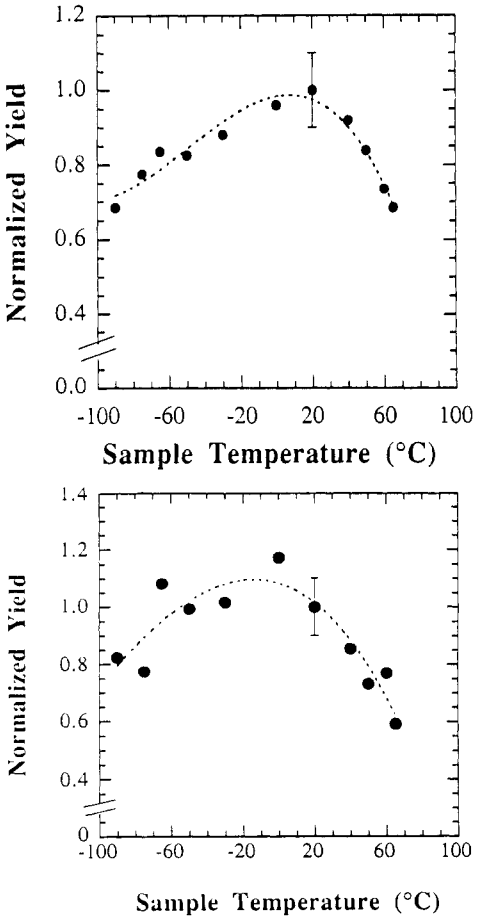

Figure 4. (a, Top) relative yield of the $[M + H]^+$ ion of insulin as a function of sample temperature. (b, Bottom) relative yield of the $[M + 2H]^{2+}$ ion of insulin as a function of sample temperature.

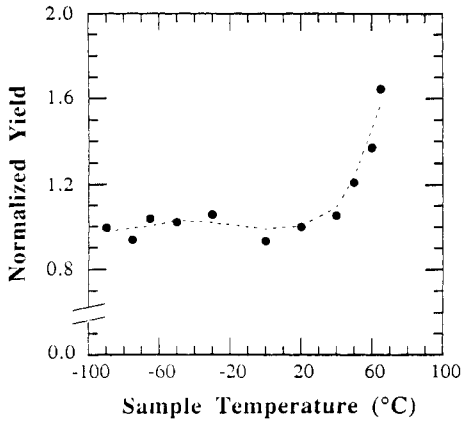

Figure 5. Relative yield of the immonium ion fragments (calculated as sum of the respective yield of ions at *m/z* 70, 72, 86, 91, 110, and 120) from insulin as a function of sample temperature.

on the electrospray mass spectra of different globular proteins.[16] The change in ion abundances and charge state distribution in that case is attributed to a transition from a more compact to a less compact conformation upon heating.[16]

The observation of a background peak at *m/z* 407 in all spectra has been a source of major concern for the validity of the data obtained upon cooling. The origin of this peak

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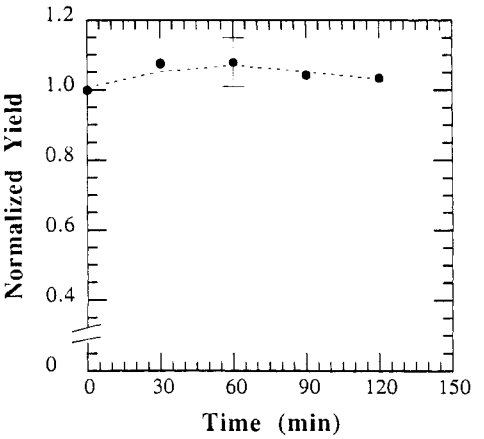

Figure 6. Time dependence of the yield of the $[M + H]^+$ ion of renin substrate at sample temperature of −50 °C.

is attributed at least partially to the oil of the diffusion pump initially used. Its intensity increases about 2 times upon cooling, but upon heating above +55 °C, it disappears completely. That peak at *m/z* 407 has not been observed at room temperature after changing the pumping system to a turbopump and employing a new target design (see above). Upon cooling to temperatures below −20 °C, a peak at *m/z* 407 appears in the spectrum even with the modified setup and with a liquid N_2 cooled Cu screen. This is an indication that an ion with the same mass may originate from condensation of volatile compounds, e.g. plasticizers in the Viton O-rings, etc. The yield of that ion is low compared to the molecular ion yield for the different samples studied. In order to assess its influence the time dependence of the $[M + H]^+$ yield for tri-ALA and RS has been monitored at −50 °C (at that temperature the yield of *m/z* 407 saturates and does not increase further upon cooling). The data (Figure 6) demonstrate that the peptide $[M + H]^+$ yield does not change with time. This proves that the observed temperature effect in PDMS of peptides upon cooling is genuine and not entirely an artefact due to residual gas condensation.

CONCLUSION

The molecular ion yield in PD mass spectra of the different peptides examined decreases both upon increasing and lowering the sample temperature. Heating leads to irreversible changes in the molecular ion yield. The dependence of the ion yield on temperature upon heating is different for peptides with different masses. Further and more systematic studies on the effect of temperature on PDMS of biomolecules may contribute to elucidation of the nature of protein–surface interactions and disclosure of the mechanisms of the electronic sputtering phenomenon per se. These studies may also result in another efficient tool for probing biomolecular conformation.

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