Mass Spectrometry

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Matrix-Free Formation of Gas-Phase Biomolecular Ions by Soft Cluster-Induced Desorption**

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Mass spectrometry of biological macromolecules has developed into a key technology for fast routine analysis in biotechnology.[1] A critical issue is the efficient transfer of nonvolatile biomolecules out of their sample solution into the gas phase in combination with their concomitant ionization. Established standard methods are matrix-assisted laser desorption and ionization (MALDI)[2] and electrospray ionization (ESI).[3] MALDI comprises laser desorption of analyte molecules that have been embedded in a matrix that is strongly absorbing at the laser wavelength;[2] in ESI, the sample solution is directly dispersed into charged nanoscopic droplets by a combination of gas injection and a strong electrostatic field applied to a microcapillary.[3] Alternative methods include massive cluster impact ionization (MCI),[4] secondary ion mass spectrometry (SIMS),[5] and electrospray droplet impact (EDI) in combination with SIMS,[6] which all make use of an impacting charged particle to desorb and ionize the biomolecules. Using a spray of charged droplets, desorption electrospray ionization (DESI)[7] combines the ESI scheme with a soft desorption process[8] and allows for mass spectrometry of biomolecules under ambient conditions.

Herein we show that neutral molecular clusters of 10^3 to 10^4 SO_2 molecules can also be used for the desorption and ionization of biomolecules. Cluster impact on arbitrary surfaces pretreated with biomolecules efficiently creates cold, desolvated, gas-phase biomolecular ions as large as 6000 u (1 u = 1 unified atomic mass unit) without any need for preparation of the biomolecules in a special matrix or means of postionization after desorption. Since the cluster provides not only the energy for the desorption process but also a transient matrix during the process, the biomolecules were found to be desorbed without any fragmentation. The time-scale on which that energy is redistributed after cluster–surface collision and biomolecule pickup is shown to be a key to the understanding of the soft desorption mechanism. Furthermore, the state of charge of the desorbed molecular ions in the gas phase can be controlled by the pH value of the original sample solution.

The experiment is sketched in Figure 1.[9,10] The SO_2 clusters are seeded in a He beam and hit the collision target under vacuum. The biomolecules have been deposited on the target by simply drop-casting the respective solution. Upon impact of the neutral cluster beam on the sample surface, the abundant formation of free molecular ions is detected with a pulsed time-of-flight (TOF) mass spectrometer, which is oriented perpendicular to the beam axis. As an example, Figure 2 shows the cationic mass spectrum from a TiN surface pretreated with a mixed oligopeptide solution. The amount of substance of each of the constituents was 10^{-10} mol. As all the spectra, it was baseline-corrected and adjusted for the mass-dependent efficiency of the microchannel plate detector.[11] The major peaks are easily assigned to the singly charged, bare oligopeptides of the original solution. Furthermore, we observe doubly charged oligopeptide ions, oligopeptide ions with SO_2 adducts, and dimers of oligopeptides. Whereas most experiments where performed with a total amount of analyte

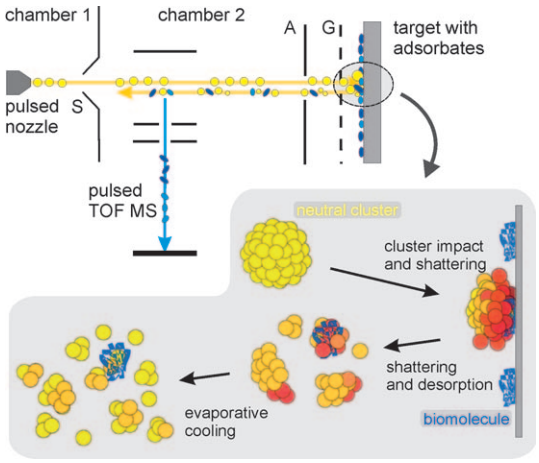

Figure 1. Upper left: Schematic depiction of the cluster-impact experiment. After the neutral cluster beam hits the sample surface, charged fragments carrying biomolecules are extracted by the biased grid (G). Mass analysis is performed in the TOF mass spectrometer oriented perpendicular to the primary beam. Skimmer (S) and aperture (A) allow for beam collimation. Lower right: schematic depictions of cluster impact and subsequent desorption of biomolecules from a surface (not to scale).

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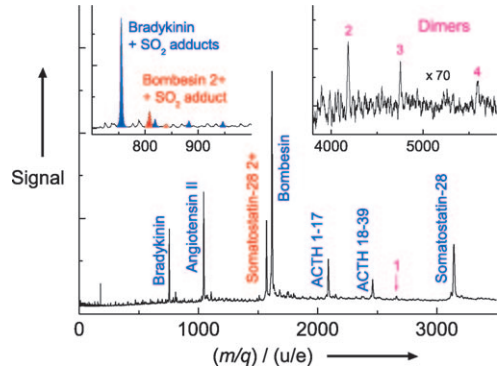

Figure 2. Mass spectrum of positive oligopeptide ions created by surface impact of SO_2 clusters with a mean cluster size of 5.7×10^3 molecules and a velocity of $v=1.5\ km\,s^{-1}$. The insets show enlargements of different parts of the spectrum. In addition to the bare, singly charged oligopeptides from the original solution, multiply charged oligopeptides, oligopeptides with SO_2 adducts, and molecular dimers are observed. The latter are labeled as follows: 1 angiotensin II/bombesin, 2 angiotensin II/somatostatin-28, 3 bombesin/somastostatin-28, and 4 ACTH 18-39/somastostatin-28.

molecules in the picomole regime, sensitivity down to the femtomole regime was demonstrated (Supporting Information, Figure S1).

The generation of multiply charged ions and its dependence on the molecular constituents of the respective oligopeptide and on the pH value is exemplified in Figure 3. Mass spectra are shown as obtained after cluster impact on a diamond surface that was covered with 1.8×10^{-10} mol insulin, 1.8×10^{-9} mol P-166, and 2.7×10^{-9} mol P-178 from aqueous solution at pH 2. Peaks can be assigned to the singly charged and twofold protonated P-166 but only singly charged P-178 are observed in the cationic spectrum. No indication of the peptides was found in the corresponding anionic spectrum (not shown). However, when the target is pretreated with a neutral solution (pH 7) containing P-178 and P-166, P-178 is

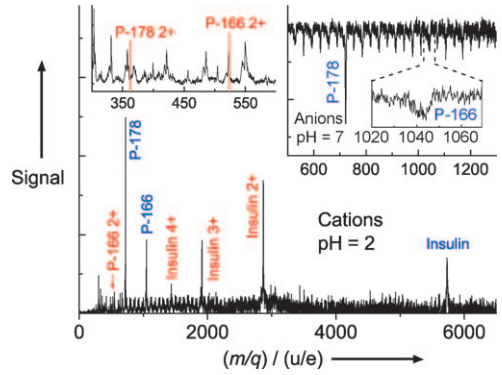

Figure 3. Main panel: Mass spectrum of positive ions generated by cluster impact on a target surface pretreated with sample solution at pH 2. Left inset: enlargement of the same spectrum. Right inset: Mass spectrum of negative ions generated from a target pretreated with sample solution at pH 7. In both the main panel and the insets, peaks from progressions of cluster fragments with a mass difference of one sulfur dioxide molecule are observable. They can be reduced in intensity, for example by increasing the initial mean cluster size or speed (cf. Figure 2 and Figure S1 in the Supporting Information).

detected in the anionic spectrum (Figure 3, right inset), but P-166 is not. Furthermore, both singly charged P-178 and P-166 ions are observed in the cationic spectrum (Supporting Information, Figure S2). Since P-166 contains arginine and histidine, two proton acceptors, formation of doubly charged ions at pH 2 is possible, but the formation of a sufficient number of negatively charged ions at pH 7 is suppressed. In contrast, P-178 contains no basic functional groups, leading to only singly charged ions even at pH 2 but allowing for a strong negative signal under neutral conditions. For the latter observation, the presence of glutamic acid, a good proton donor, may also contribute. Similar reasoning applies for the strong signal of doubly charged somatostatin-28 in Figure 2. The observed dependence of the charge state of the desorbed ions on pH value suggests a mechanism that relies on ions formed before the desorption process. The observation of positive and negative ions of one and the same species under the same experimental conditions, for example, P-178 at pH 7, however, points towards an additional ionization mechanism during cluster impact and the possibility to desorb and ionize neutral molecules. Indeed, charge transfer processes associated with cluster surface impact have been reported.[9,10,14]

The peak from singly charged insulin and a peak sequence arising from up to fourfold protonated insulin molecules are observed (Figure 3, main panel) and can again be rationalized by taking into account the pH value of the original solution (pH 2) and a fourfold presence of basic amino acids in insulin. Within the limits of experimental noise, that is, signal-to-noise ratio of approximately 100:1, no indication of the B-chain insulin fragment has been observed in the vicinity of 3.4×10^3 u. As this should be a prominent fragment ion of the singly charged insulin molecule,[12] we conclude that desorption of insulin proceeds without any fragmentation under the present experimental conditions. Delayed metastable decay can be excluded, as the desorbed molecules have to travel a few tenths of a millisecond before they are extracted into the TOF analyzer. The extremely soft nature of the desorption process is further demonstrated by the detection of peptide dimers (Figure 2). Peaks in the mass spectrum can be identified as the sum of two of the oligopeptides present in the original solution. This finding points towards the possibility that noncovalently bound molecular species can be desorbed from the surface without breaking the intermolecular bonds.

Depending on experimental parameters such as size and ratio of the kinetic energy to the total binding energy of the incoming clusters,[9,10,13] cluster progressions based on the competitive solvation of either a HSO_3^- or H_3O^+ core ion by sulfur dioxide and/or water are also observed.[14] Furthermore, progressions based on an oligopeptide with a series of sulfur dioxide adducts (Figure 2, left inset) suggest that upon impact of the cluster on the surface, the desorbed molecules are first "dissolved" in the cluster or one of its fragments produced in an early shattering process.[15] Further evaporative cooling then leads to bare or almost bare charged oligopeptides (Figure 1, bottom). This picture is substantiated by molecular dynamics (MD) simulations of the cluster collision under conditions corresponding to those of the presented experiments. Details of the calculations were described previously;[16] in short, the clusters consist of 5000

SO_2 molecules with an initial kinetic temperature of 50 K and a velocity of 1.4 km s^{-1}. The SO_2 molecules are rigid, and their atoms are treated as partially charged Lennard–Jones spheres with potentials taken from reference [17]. Their interaction with the surface is described by a simple repulsive potential.[18] The results in Figure 4 show substantial fragmentation

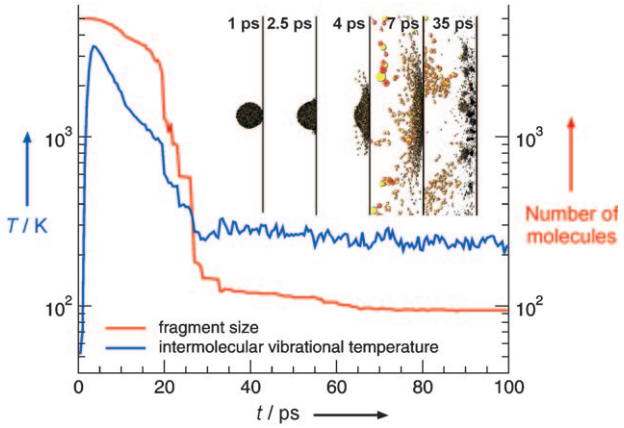

Figure 4. Kinetic temperature (left axis) and fragment size (right axis) of the momentarily largest fragment, as obtained from the MD simulation. Inset: Snapshots from a simulated collision between cluster and model surface.

of the cluster and high kinetic temperatures on the order of a few thousand Kelvin upon impact on the surface. However, these extreme conditions prevail only for a few picoseconds, as shattering of the initial cluster leads to very fast energy dissipation;[19] after only 20 ps the SO_2 cluster fragments have reached a temperature colder than the original temperature of the adsorbates. This fast energy dissipation precludes efficient energy transfer into the vibrational degrees of freedom[20] relevant for the cleavage of the relatively large biomolecules. Thus, the cluster not only provides the energy necessary for desorption through its impact onto the surface, but at the same time it helps to rapidly cool down the desorbed molecules, thereby avoiding fragmentation of the desorbed species. Driven by the remaining energy content of the desorbed macromolecule dissolved in a cluster fragment, further evaporation takes place on a nanosecond time scale and leads to the formation of almost unsolvated gas-phase analyte ions.

Finally, we briefly compare the presented neutral-cluster-induced desorption with reported desorption and ionization mechanisms. Since no permanent matrix is used for sample preparation, as is common in the case of MCI[4] and fast atom bombardment (FAB)[21] or required for MALDI,[2] molecule–matrix interaction and the contamination of mass spectra by matrix molecules is avoided. Furthermore, the cluster efficiently serves as a transient matrix, and no fragmentation is detected, in contrast to methods such as FAB[21] or cluster SIMS.[22] MCI[4] and EDI/SIMS[6] use clusters the size of ours or larger; however, they are charged, and electrostatic acceleration leads to size-independent kinetic energy per cluster. Hence, small particles of the distribution become very fast and induce surface sputtering and sample fragmentation. For example, in experiments on insulin desorption by EDI/SIMS the B and A chains appear as prominent fragments in the ion spectra.[6] In contrast, adiabatic gas expansion used herein to accelerate the neutral clusters results in a uniform velocity (i.e. clusters with constant energy density regardless of size). Consequently, sputtering and fragmentation is avoided. DESI uses charged droplets, which lead to soft desorption of multiply charged molecular ions advantageous for detection of higher masses.[7,23] In contrast, neutral-cluster-induced desorption generates ions of generally lower charge state, limiting the capabilities towards higher masses. Furthermore, it operates in vacuum, and precharged analyte molecules are preferable, similar to FAB or SIMS. However, the use of neutral clusters may reduce the generation of background ions, with a potential advantage for detection of biomolecules in the lower mass region.

Experimental Section

The SO_2 cluster beam was produced by supersonic expansion from a pulsed nozzle ($f = 10$ Hz, $t_{pulse} = 300$ μs, nominal diameter 0.3 mm) in the first chamber of a two-chamber high-vacuum setup (Figure 1). The collision target was mounted in the second chamber about 300 mm downstream from the expansion nozzle. It consisted of either a diamond- or TiN-covered silicon wafer. Transfer of the desorbed ions into the detection volume of the pulsed Wiley–McLaren-type TOF mass spectrometer was realized using a bias grid (ca. 10 lines per inch, $U = \pm 15$ V) placed 10 mm in front of the target. The TOF mass spectrometer itself was oriented perpendicular to the beam axis approximately 100 mm upstream from the target. Mass-dispersed ions were detected in a bipolar microchannel plate detector (Burle) with up to 10 kV post acceleration.

To produce clusters with sufficient kinetic energy, gas mixtures of 2.4–8.5 % SO_2 in He at a stagnation pressure of up to 15 bar have been used. The cluster beam has a narrow velocity distribution, with values ranging from of 1.2 km s^{-1} to 1.5 km s^{-1} depending on experimental conditions.[24] The beam velocities correspond to kinetic energies per SO_2 monomer between 0.5 and 0.75 eV. The cluster size distribution of the incoming beam was determined by retarding field techniques[25] after electron attachment of 9 eV electrons. The incoming cluster beam can be described by a log-normal cluster size distribution.[13]

Commercially available oligopeptides and proteins, in particular insulin from bovine pancreas (Sigma I-5500, 5733.5 u), were used (sequences and molecular masses are given in the Supporting Information, Table S1). Working solutions were based on distilled water, and, if applicable, acidity was adjusted by adding HCl(aq). Typical concentration and volume of the oligopeptide solution was in the range of several 10^{-2} mM and 1–10 μL, which equals an absolute amount of substance in the picomole regime. The respective solution was deposited on about 1 cm^2 of the target surface.

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