Gentle nano-electrospray ion source for reliable and efficient generation of microsolvated ions.

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Abstract

We present herein the design of a nano-electrospray ion source capable of reliable generation of large quantities of microsolvated ions. The source is based on a triple molecular skimmer scheme and can be quickly tuned to generate bare ions or their ionic complexes with up to more than hundred solvent molecules retained from solution. The performance of this source is illustrated by recording mass spectra for distributions of ionic complexes of protonated water, amino acids, and a small protein ubiquitin. Protonated water complexes as large as with more than 110 molecules and, for instance, of amino acids with more than 45 waters could be generated. Although the commercial ion source based on the double ion funnel design with orthogonal injection, which we used in our laboratory, is more efficient in generating ions than our triple skimmer ion source, they both exhibit comparable short-term stability in generating bare ions. In return, only the new source is capable of generating microsolvated ions.

I. INTRODUCTION

Non-covalent ionic complexes of biomolecules with solvent molecules are of great interest in a variety of structural and analytical studies that employ mass spectrometry (MS). Structural study

of microhydrated biomolecular ions allows building a bridge between their gas-phase and solution phase geometries and properties;[1-8] the complexes of biomolecules and solution tags (e.g., molecules of solvent, soluble aromatics) can be used for spectroscopic identifications of isomers;[9-11] study of protonated clusters of water molecules sheds light on structure of liquid water,[12,13] etc. Despite many decades of research, reliable generation of large quantities of microsolvated biomolecular ions still remains technically a difficult task.[14,15] There are two common approaches for production of weakly bound charged molecular complexes. In one of them, charged biomolecules are, first, produced by electrospray ionization (ESI) in the gas phase as fully desolvated species. These bare ions are then trapped and collisionally cooled in a cryogenic ion trap, to which a desired solvent in the form of vapor is injected through a gas valve. The vapor condenses onto the cold ions, producing charged microsolvated biomolecules with different number of non-covalently bound solvent molecules.[7,14,16,17] While technically relatively simple, the method of cryogenic condensation cannot ensure that rehydration of a fully desolvated biomolecule will reshape it back from intrinsic to native structure, unless the structure is "kinetically" trapped.[14] On technical side, the injected vapor not only unavoidably condenses onto the ions, but also freezes onto electrodes of cryotraps. The formed non-conductive patches may charge and distort performance of the trap, ultimately requiring interruption of measurements and heating the trap to evaporate the solvent.

A more "natural" approach for producing non-covalent ionic complexes is to transfer them directly from solution phase using a sufficiently "gentle" ESI process.[2,13,15,18] Retaining a few water molecules on a biomolecule should allow for preserving of, at least, the main features of its native structures. Despite a few decades of studying microsolvated biomolecules and protonated water clusters, construction of a robust and efficient ESI source capable of reliable production of

large quantities of such non-covalent complexes directly from solution still remains problematic. There is a subtle balance to be found and maintained between full desolvation and lack of ionization for a reliable generation of large quantities of microsolvated ions. In most common gentle ESI sources ions enter a vacuum chamber from atmosphere through a few cm long heated metal capillary of less than 1 mm internal diameter and subsequently pass through one or two closely spaced consecutive molecular skimmers, which work as conductance limits for differentially pumped sections of the chamber. This arrangement enables sharp drop of pressure over short pathway of ions to minimize energy and number of collisions during transportation of ions from the exit of the capillary to the high vacuum part of the chamber. We previously employed such a source, which was based on a long glass capillary and single skimmer.[2,19] The source could produce microhydrated complexes of, for instance, a doubly protonated decapeptide with as many as 50 water molecules.[3] Despite a series of successful experiments, our experience with such a source revealed its low stability and productivity, as well as high dependence of these characteristics on humidity in the laboratory. For still not fully understandable reasons, a good distribution of clusters could be generated on rainy days only, while a use of various humidifiers did not help.

Here we report the design of a nano-ESI source that exhibits high stability and good efficiency in generating small to large microhydrated clusters of different charged biomolecules directly from solution by retaining water molecules. The performance of the source is illustrated by mass spectra of the generated distributions of microhydrated complexes. We compare some key characteristics of this gentle source with those of a commercial one based on electrodynamic ion funnels.

II. INSTRUMENTATION

Figure 1 shows the cross section of the vacuum chamber that accommodates the gentle ion source. The main feature of the chamber is the use of three differentially pumped sections separated by three inline closely spaced molecular skimmers. This arrangement allows efficient transportation of ions in the gas flow from the atmospheric pressure ionization region to an RF octupole ion trap, which operates at pressure of ~10^{-4} mBar, over the distance as short as ~125 mm. The ions are generated from solution by nano-ESI (Proxeon) that uses metal coated borosilicate emitters (Thermo Fisher; emitters with "long" tip). Depending on the cut of the tip, the typical flow of solution through these emitters is 50 to 150 nL/min with $5 \cdot 10^{-5}$ molar concentration of biomolecules. The ions are transferred from atmosphere to the first section of the source vacuum chamber through a 10 cm long stainless steel (SS) capillary of 0.5 mm I.D. All sections of the chamber are made of aluminium and vacuum-sealed together with Viton® O-rings. The capillary is mounted in a SS block, which also contains a 50 W heater cartridge and a thermocouple to control temperature of the block within 20-170° C. A 35 m^3/h oil mechanical pump allows for maintaining the pressure of 2.5 mBar in this section. The exit of the capillary is axially aligned with the orifice of the first molecular skimmer, which separates the 1st and the 2nd sections of the source. The skimmer is a thin SS cone with the opening angle of 90° and the orifice of 1 mm diameter. The second section is pumped to 0.25 mBar pressure by a 35 m^3/h dry scroll pump. Downstream, this section is limited by the 2nd molecular skimmer, glued at the base to the supporting aluminium cylinder; the glue (vacuum epoxy) also serves for insulating the skimmer electrically. The 2nd skimmer (Beam Dynamics) has 2 mm diameter of the orifice and is placed 8 mm downstream from the orifice of the 1st skimmer. The 3rd vacuum section is limited by the 3rd skimmer (3 mm diameter of the orifice, Beam Dynamics), which is glued on top of an aluminium cone. The distance between the openings of the 2nd and 3rd skimmers is set to 15 mm, but varying

it within ±2 mm appears to be not critical for the overall performance of the source. This section is pumped to the pressure of $5 \cdot 10^{-3}$ mBar by a 260 L/s turbomolecular pump backed by a 30 m^3/h mechanical pump. The ions that pass through the orifice of the 3rd skimmer enter to a 26 cm long octupole ion guide, where they can be, optionally, accumulated to increase their total number. This final octupole section of the source is equipped with a 67 l/s turbopump mounted on a tube that crosses through the 3rd section of the source. To accumulate ions, the potential of the octupole exit endcap is raised by 10-15V above the pole bias of the ion guide for the period of accumulation. The trap opens by lowering the potential of the endcap to 1-2 V below the pole bias. The released ions are guided to a quadrupole mass filter and, finally, the m/z-selected ions are detected by a channeltron and counted by a gated photon counter.

The distance of 2-3 mm between the capillary and the first skimmer appears to be optimal to balance the maximum size of microhydrated complexes and the total ion current. Shortening this distance sharply induces desolvation of ions, while increasing this spacing leads to a rapid drop of the ion current. The distribution of microsolvated ions is largely controlled by temperature of the capillary and by its electric potential relative to the 1st skimmer. Raising this voltage slightly increases the total ion current but shifts the distribution of complexes to smaller sizes. This operation mode of the source can be used whenever retaining solvent molecules on ions is undesired. Although the ion current (or the number of trapped ions, when the pre-trap is engaged) is stable even under dry atmosphere of our air-conditioned laboratory (relative humidity <20%), an elevated local air humidity in the volume between the emitter and the entrance capillary yet improves the stability of microhydrated ions. It is interesting that only the commercial humidifiers that are based on room-temperature “drying” of wet paper filters work well; neither ultrasonic-based or heat-assisted evaporative humidifiers do not improve generation of microhydrated ions.

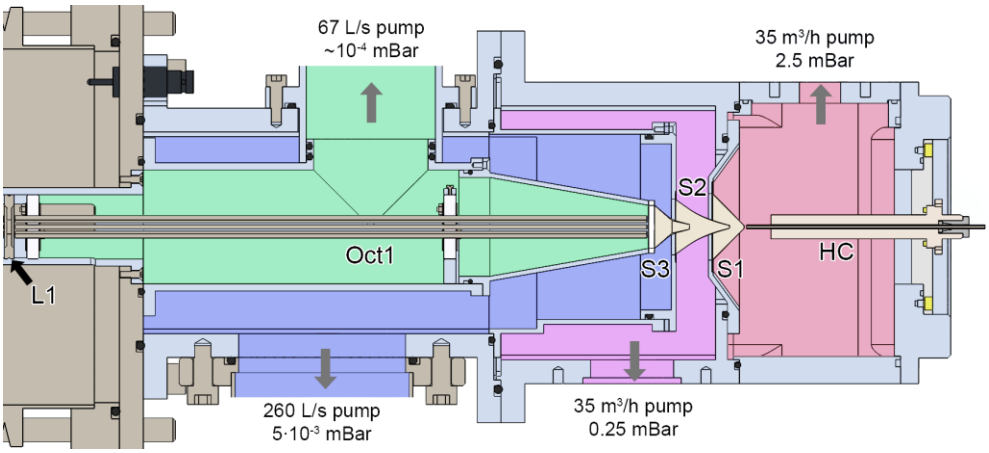

FIG. 1. Cross-section of the "gentle" ion source. Four differentially pumped sections of the source vacuum chamber are differently coloured. HC–heated capillary, S1, S2, S3 –molecular skimmers with the orifice diameters of 1, 2 and 3 mm, respectively; Oct1 –octupole ion guide/trap, L1– endcap electrode.

III. EXPERIMENTAL

Figure 2 shows mass spectra of protonated water complexes that are produced by new gentle ESI source from LC-MS grade water with 0.2% of acetic acid in the continuous mode of operation (no pre-trapping in the octupole ion guide). In these four measurements all settings of the source were kept the same, except the capillary voltage and temperature. These two parameters appeared to be the main to control "gentleness" of the process. Fig. 2a shows the distribution of the protonated water complexes at the capillary temperature and voltage of T=150° C and V=60 V, respectively. With these values the source becomes relatively harsh, such that only small complexes with the number of water molecules n=4-21 are generated. Lowering the capillary temperature to T=50° C widens distribution of the clusters (Fig. 2b), which spans now from n≈11 to n≈100 with the characteristic "magic" number n=21.[20] The increase of the temperature of the capillary to T=80° C and lowering the potential to V=40 V narrow the distribution, but shift its maximum towards larger sizes of the clusters (Fig. 2c). At the softest conditions that allow stable generation of large quantities of protonated water clusters (T=50° C, V=40 V) the distribution further shifts toward

very large complexes (Fig. 2d). The largest observed clusters contain n~110 water molecules (m/z=1980 Th); larger clusters, apparently, are generated too, but cannot be detected due to the upper m/z limit of 2000 Th for transmittance of our quadrupole mass filter. Overall, the average size of the clusters can be controlled by both parameters, although the width of the distribution is more sensitive to the temperature of the capillary. Other parameters, such as, for instance, skimmer voltages and amplitude of RF waveforms applied to the ion guide have a minor influence on the harshness of the ion source. It is worth mentioning that the total number of the generated ions remained, roughly, almost the same for all the settings in Figure 2 with a slight increase for higher voltage on the capillary. Maximizing ion signal for one particular size of the clusters with low number of water molecules requires an increase of the capillary temperature and potential. We attribute this to the consecutive dissociation of the larger clusters, which feed the population of the smaller ones.

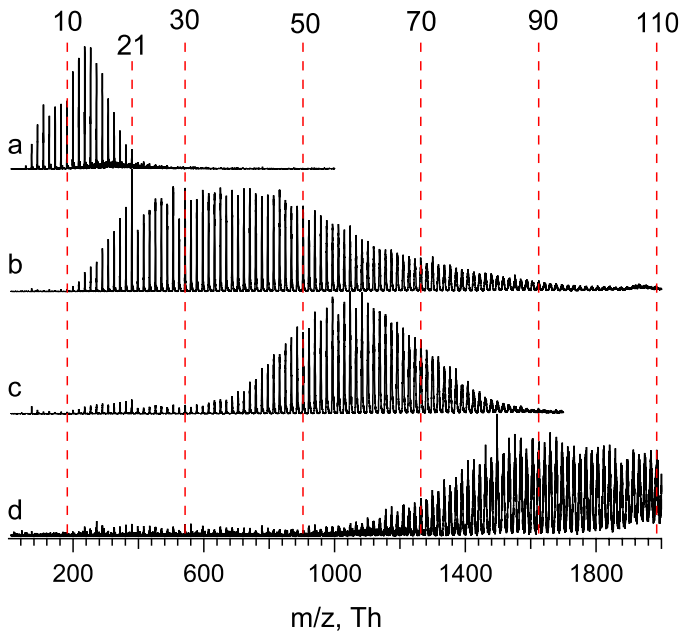

FIG. 2. Mass spectra of protonated water complexes $(H_2O)_n$-H^+generated by the nano-ESI source at the capillary temperature and the voltage of (a) 150 °C and 60 V, (b) 50 °C and 150 V, (c) 80 °C and 40 V,

(d) 50 °C and 40 V. The numbers on top indicate the number ***n*** of water molecules in the mass peaks aligned with respective vertical dashed red lines. Ions were not trapped in the octupole ion guide.

As an example, Figure 3 shows the distributions of microhydrated His-H^+ complexes generated by our nano-ESI source at temperature of 130° C and different capillary voltages. Lowering the capillary voltage gradually changes the most intense peak in the distribution from the bare ion, $HisH^+$ (Figure 3a), to the $HisH^+–(H_2O)_{25}$ complex (Figure 3e). The minor distributions in the spectra correspond to the protonated and sodiated water complexes, which are typically present in mass spectra of microsolvated biomolecules.

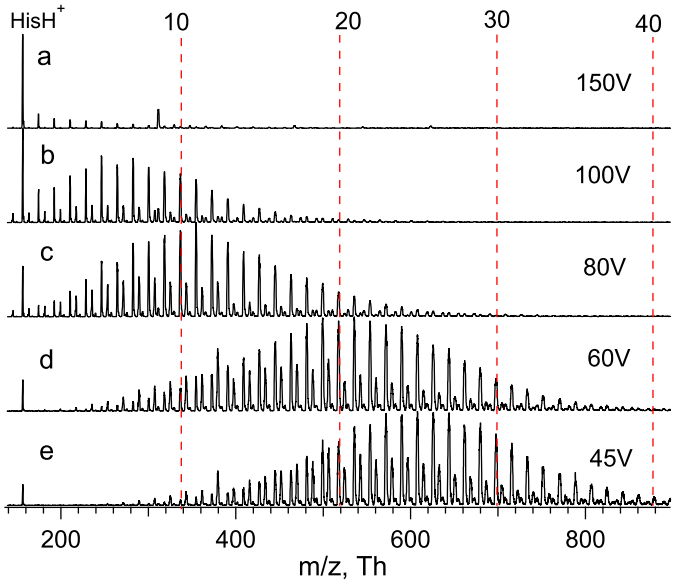

FIG. 3. Mass spectra of microhydrated protonated amino acid $HisH^+–(H_2O)_n$ that are generated by the triple-skimmer nano-ESI source with different potentials of the metal capillary (labelled on the right). The numbers on top indicate the number *n* of water molecules in the mass peaks that are aligned with the respective vertical dashed red lines. Ions were not trapped in the octupole ion guide/trap.

Large quantities of complexes with single to several retained water molecules have been generated with the new source for larger ions such as protonated tripeptide Gly_3H^+,[5] pentapeptide

enkephalin (see SI) and even for a small protein ubiquitin.[6] In addition to water, other solvent molecules, such as methanol, acetonitrile, and 2-butanol could be retained on carbohydrates (the former three solvents) and on lipids (the latter solvent) using gentle mode of operation of the source.[11] Ultimately, by raising the capillary temperature and potential the source can be tuned to a standard, harsh, mode of operation, to generate only fully desolvated positive ions in large quantities.

In our spectroscopic experiments, we, typically, accumulate and thermalize ions by pre-trapping them at room temperature in the octupole ion guide for almost 50 ms prior transferring the ions into a cold ion trap for performing photofragmentation. This maximum duration of pre-trapping is determined by the repetition rate of the lasers used for photofragmentation. Figure 4 compares the size distributions of microhydrated complexes of amino acid $ArgH^+$ with and without this accumulation. The relative abundances of the complexes were derived from the respective mass spectra. Without accumulation, the distribution of the complexes appears to be broad with the maximum at $ArgH^+$ $(H_2O)_{12}$ and with 12% abundance of the fully dehydrated amino acid. Trapping results in evaporation of water from the complexes, which are metastable at room temperature, such that after 50 ms of the accumulation large complexes become almost fully suppressed and the whole distribution shifts towards smaller clusters. Notably, the abundance of small (n=1-3) complexes increases significantly, which makes pre-trapping an advantage in studies of small complexes. In addition, the accumulation substantially reduces the fluctuations of the number of ions that can be finally trapped in the cold ion trap.

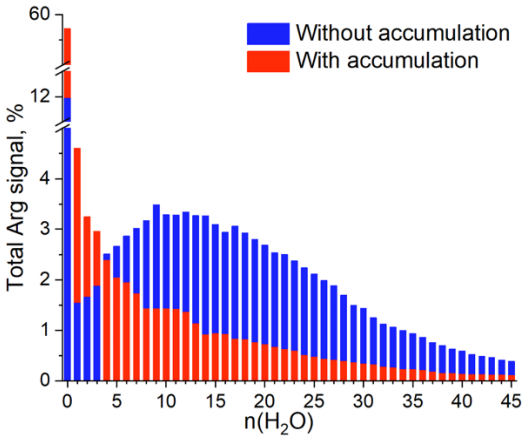

FIG 4. Distributions of $ArgH^+–(H_2O)_n$ non-covalent complexes measured with and without accumulation for almost 50 ms in the octupole ion guide.

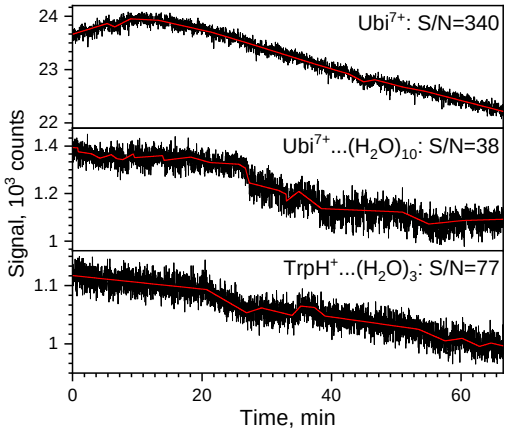

FIG. 5. Typical time evolution of the number of parent ions generated by the new triple-skimmer nano-ESI source and counted within 3 ms time-gate in spectroscopic experiments with (from top to bottom) protein ubiquitin in +7 charged state (Ubi^{7+}), with $Ubi^7–(H_2O)_{10}$ complex and with microhydrated amino acid $TrpH^+$-$(H_2O)_3$. Each data point is an average of 10 measurements; each trace (black lines) is labeled by signal to noise ratio calculated with respect to the average (red lines).

Figure 5 illustrates the level and time stability of ion signals in our typical spectroscopic experiments with the triple-skimmer ion source. In all the measurements the source settings were optimized to maximize generation the ions of interest. In the experiments with fully desolvated

protein ubiquitin in the +7 charge-state, the ion signal appears to be only about 25% less with the new source, than with the double ion funnel (DIF) ESI source that we previously used with the same instrument. For smaller ions (e.g., singly protonated amino acids), the efficiency of the triple skimmer ion source becomes, however, 30-60% lower than that for the DIF ESI source. The measured ion signals (Figure 5) drop by 15-20 times when the new source is optimized for maximum efficiency in generating $Ubi^{7+}(H_2O)_{10}$ and $TrpH^+$-$(H_2O)_3$ complexes, although, as we mentioned above, the integral ion signal for each distribution of the complexes remained nearly the same as the signals in the experiments optimized for maximum signals of the respective fully desolvated ions. It is worth mentioning that only monomers of the bare and mycrohydrated protein could be form under the conditions of our experiments.[6] The short-term stability of the new source is quite high and comparable with our DIF ESI source in generating fully desolvated ions. It reduces however by 9 and 4.5 times for the microhydrated complexes of Ubi^{7+} and of $TrpH^+$, respectively (Figure 5). This higher instability reflects the subtle balance that is to be find every time between softness and efficiency of the source. We attribute the mid- and long-term changes of the signals in Figure 5 to typical instabilities of the solution flow through the glass capillary or else of the electric discharge in the ionization region of our nano-ESI sources. Finally, it is worth noting that all our efforts to generate any water complexes with DIF ESI source or with a combination of one funnel and one/two skimmers have failed. Our intermediate and simpler designed, which was based on two consequtives skimmers, demonstrated a moderate stability, but an order of magnitude lower efficiency in generating micro solvated complexes.

In conclusion, we designed and tested a nano-ESI source, which is based on a triple skimmer configuration. The source can be quickly tuned to a “gentle” mode of operation for generation of large number of microhydrated ions with, for instance, more than hundred retained

water molecules, but also to a standard "harsh" mode of operation to generate fully desolvated ions. In comparison with our previous double ion funnel nano-ESI source, which could not produce any ion-solvent complexes, the new source is less efficient in generating fully desolvated ions, but demonstrates a good short-term stability in generating both bare protonated biomolecules and their microhydrated complexes. Such gentle ion source can be used not only for spectroscopy of microhydrated biomolecules, but, potentially, also in native mass spectrometry of proteins and protein complexes. We earlier spectroscopically shown for protonated ubiquitin in the charge states +7 to +9 that even a few water molecules retained on the electrosprayed proteins preserve their unfolded native-like structures.[6] Continuous evaporation of weakly bound water molecules protects the ions from an excessive heating during and after ESI, which may lead to unfolding of the protein. A biomolecule that is electrosprayed from solution to the gas phase with a few waters has a good chance to retain its native structure on the time scale that is sufficient for MS interrogations.

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