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Evaporation of the charged droplets in the heating flow tube under atmospheric pressure: observation of the H/D exchange and supermetallization

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Gas phase ion-molecular reactions, which have been performed under atmospheric pressure and elevated temperature, include H/D exchange, conformational changes, and supermetallization.

The electrospray ionization (ESI) approach developed by Fenn[1] is now one of the most widely used soft ionization techniques in the mass spectrometry. In the ESI ionization source the solution flows through the thin capillary that is kept under the high voltage. At the capillary tip the solution forms the Taylor cone and charged droplets emit from the tip of the Taylor cone. The droplets evaporate, shrink and, when the Columb repulsion force exceeds the surface tension force, the droplets reduce their charge by emitting numerous charged microdroplets from the surface.[2,3] A repetition of this process results in the formation of the gas phase ions of the investigated sample. Shortly after the discovery of the ESI it was recognized that the capabilities of the ESI source can be improved by the facilitation of the droplet evaporation in the heated ion guide.[4,5] Currently all commercial ESI sources use the evaporation of the droplets under the elevated temperature. The application of the high temperature in the ESI source enables an accomplishment of the gas phase ion molecular reaction under atmospheric pressure. By infusing the reagent gas into the ESI source ions can be subjected to ozonolysis,[6,7] hydrogene/deuterium exchange (H/D exchange)[8–17] (including exchange of some CH hydrogens[18–20]), $^{16}O/^{18}O$ exchange,[21] and the conformational changes.[11,13,14] Also, at high temperature the formation of the ion peptide–metals complex, when the peptide accepts unexpectedly large number of metals, can occur. We have called this effect the 'supermetallization'.[22,23] To observe almost all of such effects, high temperature (~400 °C) of the ion transfer capillary is required. Such temperature cannot be reached on many commercial mass spectrometers. So, it is important to develop the approach for performing gas phase ion molecular reaction outside of the ESI source and the ion transfer capillary of the mass spectrometer.

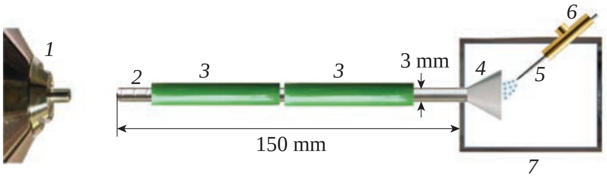

Figure 1 The flow tube developed: (*1*) mass spectrometer entrance, (*2*) metal tube, (*3*) heating element, (*4*) funnel, (*5*) electrospray needle, (*6*) interface for the introduction of the auxiliary gas, (*7*) ESI chamber.

Here we present the design of the heating flow tube that makes possible the evaporation of the charged droplets under high temperature and atmospheric pressure. The design of the flow tube is shown in Figure 1. The solution is delivered through the ESI needle to the hermetic chamber. The needle is kept under voltage of 3 kV and charged droplets are produced at the tip of the needle. The ESI needle is blowed by the auxiliary gas. This gas can be ambient air, or can contain the vapors of D_2O in order to perform the gas phase H/D exchange reaction. The pressure in the chamber pushes the droplets through the metal tube to the atmosphere. The droplets evaporate inside the tube heated to high temperature. The inner diameter of the tube was 3 mm, the length of the tube was 150 mm, the open end of the tube was positioned 40 mm from the mass spectrometer entrance.

Gas phase H/D exchange that occurs during the ESI is the simplest example of the gas phase ion molecular reaction that depends on the temperature. The results of the gas phase H/D exchange of dihydroxyphenylalanine (DOPA)[†] are presented in Figure 2(*a*),(*b*). Protonated DOPA ion possesses six labile hydrogen

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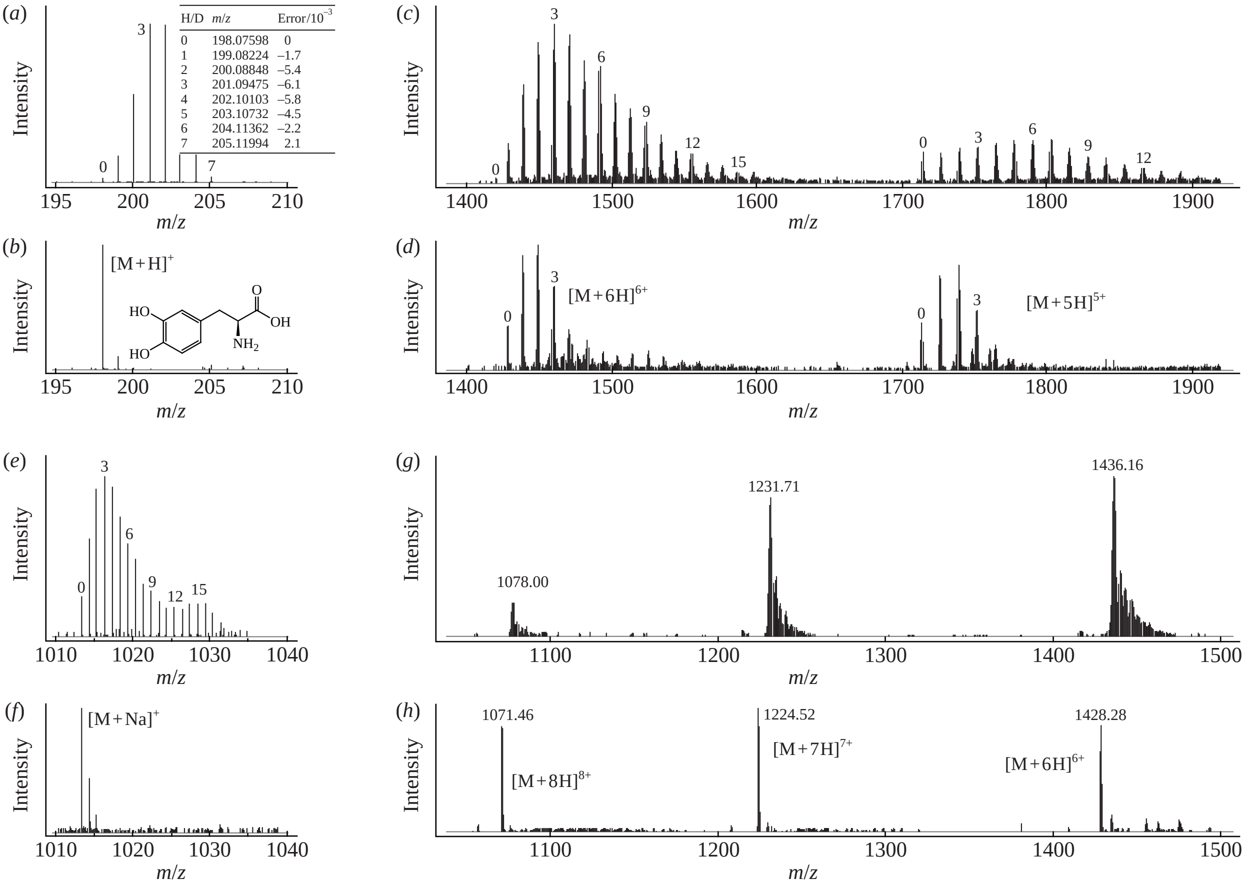
[†] *Sample preparation.* The solution of dextran was prepared in the concentration 1g dm^{-3} in a mixture of water and methanol (1:1) with the addition of AcONa (1 mM). The ubiquitin solution was prepared in the concentration 0.1 mmol dm^{-3} in the same solvent with $Zn(AcO)_2$ (10 mM) and formic acid (1%). The DOPA solution was prepared in the water–methanol (1:1) mixture. All chemicals were of analytical grade.

MS analysis. All experiments were performed using a LTQ FT Ultra (Thermo Electron Corp., Bremen, Germany) mass spectrometer equipped with a 7T superconducting magnet. The ions were generated by an IonMax Electrospray ion source (Thermo Electron Corp., Bremen, Germany) in a positive ESI mode. The temperature of the desolvating capillary was set to 50 °C. The length of the desolvating capillary was 105 mm and its inner diameter was 0.5 mm.



H/D	m/z	Error/10^{-3}
0	198.07598	0
1	199.08224	–1.7
2	200.08848	–5.4
3	201.09475	–6.1
4	202.10103	–5.8
5	203.10732	–4.5
6	204.11362	–2.2
7	205.11994	2.1

Figure 2 The results of the experiments. (*a*) DOPA in D_2O atmosphere at 420 °C, (*b*) DOPA; ubiquitin with $Zn(OAc)_2$ at (*c*) 500 °C, (*d*) 120 °C; (*e*) dextran $H(C_6H_{10}O_5)_6OHNa$ in D_2O atmosphere at 400 °C, (*f*) dextran $H(C_6H_{10}O_5)_6OHNa$; (*g*) ubiquitin in D_2O atmosphere at 350 °C, (*h*) ubiquitin.

atoms and they all are exchanged for deuterium. In addition, under the elevated temperature of the flow tube it is possible to observe the exchange of the CH hydrogens for deuterium. The fact that the peaks labeled '7' in Figure 2(*a*) indeed correspond to the 7 H/D exchanges is proven by the ultrahigh resolution FT ICR.[24,25] The peaks corresponding to the H/D exchange reaction have mass difference 1.006277 Da (this is the difference between masses of D and H). The difference between measured and theoretical m/z of the peak corresponding to 7 exchanges is 2.1×10^{-5} Da.

The supermetallization is the recently observed phenomenon of the formation of the peptide–metal ions complex in the gas phase when the peptide accepts unexpectedly large number of metals.[22,23,26] It was found that the supermetallization occurs during the electrospray ionization when charged droplets are evaporated under relatively high temperature (~400 °C). The masses of the supermetallized ions obey the equation:

$$M_{\mathrm{complex}} = M_{\mathrm{peptide}} + nM_{\mathrm{metal}} - V_{\mathrm{metal}}\,n\,M_{\mathrm{H}} + z\,M_{\mathrm{H}},$$

where M_{metal}, M_{H}, M_{peptide} are the masses of metal, hydrogen, and the peptide; n is the number of metal adducts, V_{metal} is the valence of the metal, M_{complex} is the mass of the formed complex and z is the charge. In Figure 2(*c*),(*d*) the FT ICR spectra of complexes of ubiquitin with Zn^{II} obtained for different temperatures in the flow tube are depicted. When the temperature of the flow tube is increased to 500 °C the supermetallized complexes of ubiquitin with up to 15 Zn atoms are observed. The supermetallization was detected for charged states 5+ and 6+.

In our previous work,[12,13] we have observed that oligosaccharide cations and anions produce bimodal deuterium distribution during H/D exchange in the ESI source. Other investigated objects (peptides, proteins, low molecular organic compounds) have never demonstrated the bimodal shape of the deuterium distribution. The effect has been explained by the assumption that the flexibility of the oligosaccharide allows folded and unfolded conformations to form in the gas phase during the ionization. We have successfully reproduced the bimodal deuterium distribution for dextran $H(C_6H_{10}O_5)_nOH$,† which is the complex glycan composed of glucose molecules. Neutral molecule of dextran possesses $3n + 2$ labile hydrogens. Here n is the number of glucose residues in the molecule. In order to obtain positively charged ions of dextran we have added AcONa to the solution. In this case, dextran is ionized forming $[H(C_6H_{10}O_5)_nOH + Na]^+$ ions. The spectrum of the $[H(C_6H_{10}O_5)_6OH + Na]^+$ subjected to the gas phase H/D exchange is shown in Figure 2(*e*). It is evident that this ion produces bimodal deuterium distribution indicating the presence of two differently folded gas phase conformations ('folded' and 'unfolded') with different deuterium exchange rate.

Previously we have investigated the H/D exchange in the ESI source under atmospheric pressure for peptides and proteins of different molecular weight.[11,14] Using the flow tube developed here, we have performed the gas phase H/D exchange of the small protein ubiquitin.† Gas phase conformations of ubiquitin were previously studied in detail.[11] Lower charge states with a smaller cross section are associated with the folded conformations and higher charge states with a larger cross section are associated with the unfolded ones. These conformations are capable of converting from one to another.[27] Collisional cross section (CCS) of gas phase ions of ubiquitin varies from 1460 Å^2 (z = +7) to 2220 Å^2 (z = +11). Ubiquitin possesses totally 144 labile hydrogens: 72 of hydrogens are in the main chain and 72 of hydrogens are in the back bone. Figure 2(*g*) shows the results of the gas phase H/D exchange for the charge states 8+, 7+, and

6+. We can see that ions $[M + 8H]^{8+}$, $[M + 7H]^{7+}$, and $[M + 6H]^{6+}$ demonstrate 52, 50, and 47 exchanges, respectively. These results are in agreement with the previous experiments.[11]

Thus, we have demonstrated the design and operation of the heated flow tube that makes possible to perform ion molecular reactions under elevated temperatures and atmospheric pressure. The system emits the products into atmosphere, where they can be analyzed using any commercial mass spectrometer. In addition, it may be possible to deposit the formed molecular ions on the target and analyze their structure using X-ray diffraction or electron microscopy.

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