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Supermetallization of Substance P during electrospray ionization

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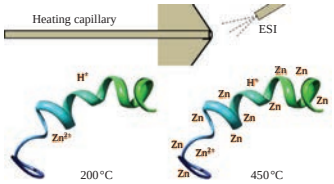
The formation of the gas phase complexes of a peptide that does not contain His and Cys residues and OH groups with up to six Zn atoms during electrospray ionization was observed.



The metal–peptide complexes play a critical role in the progression of neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD) and prion diseases (TSEs).[1] High concentrations of copper and zinc occur in amyloid plaques from AD brains and in the cerebrospinal fluid (CSF) of patients affected by both AD and PD. Proteins associated with neurodegenerative diseases bind several transition metal ions, and metal binding results in the aggregation of these proteins. It is well known that, at high pH values, a metal ion can substitute the hydrogen atom of the amide nitrogen. Such a process is facilitated by the anchoring of the metal ion in order to chelate to the amide oxygen.[2] It is believed that transition metals such as Zn^{2+}, Co^{2+}, Ni^{2+} and Cu^{2+} are chelated mainly to only the amino acids histidine and cysteine. It was also demonstrated that reducing the pH of the system below the pK_a of the binding site leads to the protonation of this site and the reducing of metal coordination.[3,4] The Zn complex of amyloid beta peptide is one of the most biologically important metal peptide complexes.[5,6] The binding of this peptide to Zn results in the formation of amyloid plaques and leads to the progression of the AD.[7,8] For the investigation of the interaction of peptides with metals, isothermal titration calorimetry (ITC), EPR, NMR, IR spectroscopy, X-ray absorption spectroscopy (XAS), electrochemical methods, and mass spectrometry are used.[9] In contrast to other methods, which provide information on native peptides, mass spectrometry deals with gas phase ions formed upon electrospray ionization (ESI), matrix assisted laser desorption ionization (MALDI), *etc*. The problem of the extent to which the structure of gas phase ions reflects the solution phase structure is still under consideration. Only a few times, the question of the possibility of metal deposition during ionization processes was raised.[10,11] On the other hand, amino acid complexes with transition metals in a gas phase are of considerable interest.[12–14] Unitary and binary complexes of Cu with all amino acids including optically active amino acids[15] were investigated.[16] Upon electrospray ionization, the ions $[M-nH+mX]^{(m-n)+}$ (where X is an alkali metal ion) can be formed.[17,18] Recently, we found that such an effect is common for any metal ions, and it considerably depends on the desolvating capillary temperature of the ESI source. For example, the (1-16) Amyloid peptide, which has three Hys residues, forms a complex with eleven Zn atoms and each Zn substituted two hydrogen atoms.[19] We refer to this effect as supermetallization. Here, we studied the peptide Substance P [Figure 1(*a*)], which does not have His and Cys residues and OH groups; nevertheless, it can be supermetallated by Zn.

Charged droplets of a solution of the peptide and a zinc salt are produced by ESI. These charged droplets passed through a heating metal capillary, evaporated and produced ions [Figure 1(*b*)], which were measured by a mass spectrometer.[†] The desolvating capillary temperature affects the droplet evaporation dynamics and hence the formation of gas phase ions. The desolvating capillary temperature plays the major role for controlling the in-ESI source gas-phase reactions.[20–31]

We observed that the metal peptide complexes were formed if the capillary was heated to 450 °C. This effect is unexpected because the high temperature should destroy all weakly bound complexes, and metal–peptide complexes are formed *via* non-covalent coordination of Zn by several amino acids (usually, His or Cys). The formation of complexes at a high temperature allowed us to suggest that they are produced *via* a different mechanism. Indeed, the use of high resolution Fourier transform

[†] *Sample preparation*. The samples were dissolved in a 1:1 mixture of water and methanol with the addition of $Zn(AcO)_2$ and 1% formic acid. The peptide concentration was 50 μM, and the concentration of Zn ions was 100 times higher. We used the peptide Substance P (RPKPQQFFGLM with an amidation at the C-terminus). All chemicals were of analytical grade.

MS analysis. All experiments were performed on a LTQ FT Ultra (Thermo Electron Corp., Germany) mass spectrometer equipped with a 7T superconducting magnet. Ions were generated by an IonMax Electrospray ion source (Thermo Electron Corp.) in a positive ESI mode. The desolvating capillary temperature was varied from 50 to 450 °C. The length of the desolvating capillary was 105 mm, and its inner diameter was 0.5 mm. The sample infusion rate was 1 μl min^{-1}, and the needle voltage was 3000 V.

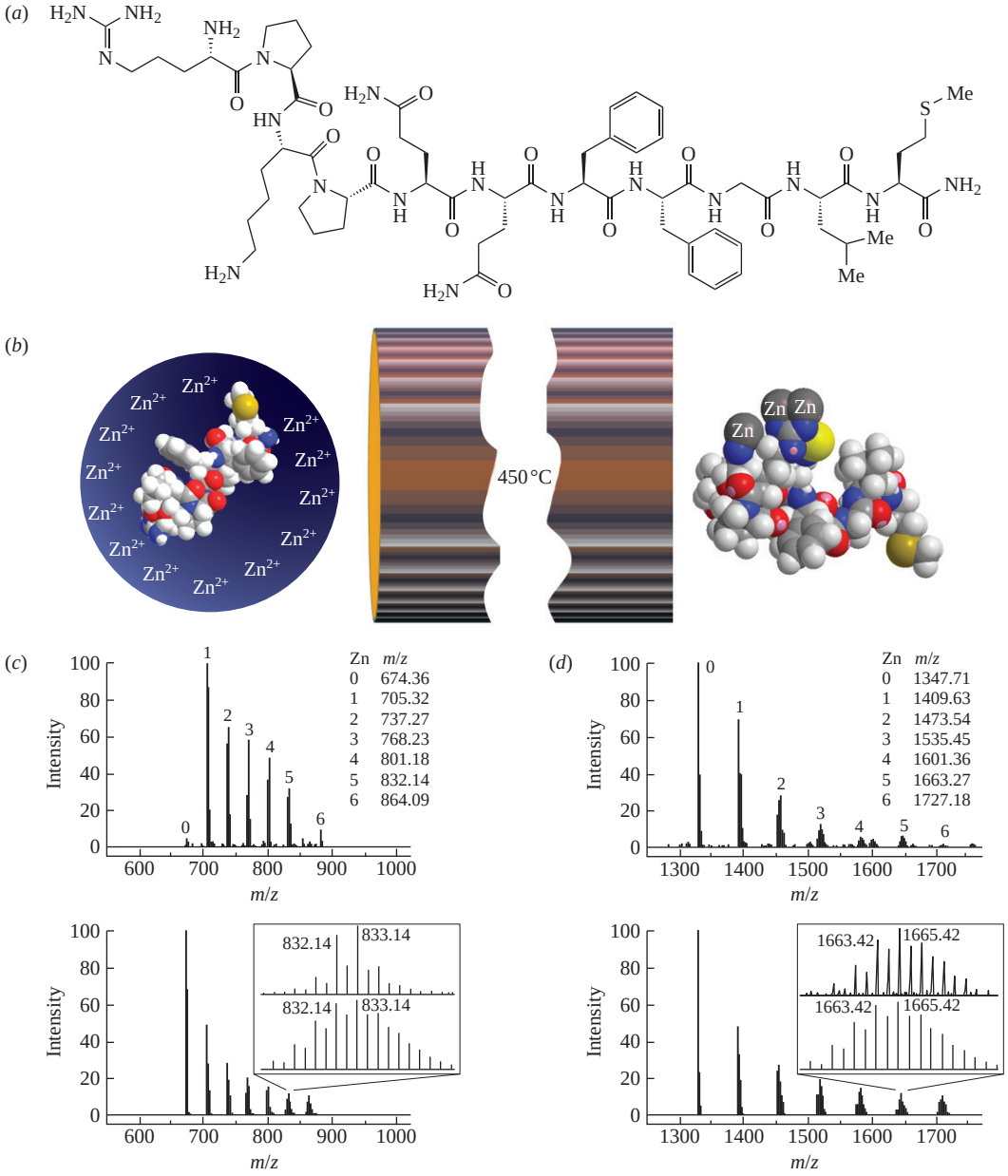


Figure 1 (*a*) The structure of Substance P. (*b*) The design of the experiment. Charged droplets evaporate inside the heated capillary producing peptide complexes with zinc. (*c*), (*d*) Measured (top) and simulated (bottom) mass spectra for the charge states 2+ and 1+. Inserts: measured (top) and simulated (bottom) isotopic distributions within the single complex.

ion cyclotron resonance (FT ICR) demonstrates that the masses of Zn adducts obey the equation

$$M_{complex} = M_{peptide} + n M_{Zn} - 2n MH + z M_H,$$

where M_{Zn}, M_H and $M_{peptide}$ are the masses of zinc, hydrogen and the peptide, respectively; n is the number of Zn adducts, $M_{complex}$ is the mass of the formed complex, and z is the charge. Such a relationship was proved by the coincidence of the measured and simulated isotopic distributions [Figure 1(*c*),(*d*)]. Therefore, the formation of a complex occurs *via* the replacement of two hydrogen atoms by a Zn atom.

We used electron capture dissociation (ECD) and collision induced dissociation (CID) for studying the structure of complexes. All the adduct peaks of the secondary charged Substance P were fragmented, and fragments were identified. Figure 2 shows that, with increasing the number of Zn atoms in the complex, the fewer fragment ions can be observed. Such results are well demonstrated for the case of ECD fragmentation [Figure 2(*b*)]. The secondary charged Substance P ion produces a series of singly charged ions from c_4 to singly charged Substance P. With increasing the number of zinc, short c-ions can no longer be detected and, for the case of six zinc atoms, only a singly charged complex is observed. These results indicate that zinc atoms bound to different amino acids residues create the strong frame that binds and stabilizes the molecule. This is consistent with the size and geometry parameters of the Zn ion, which is too big to form double bound with one nitrogen atom. Similar results were obtained for the CID fragmentation. With the increase in the quantity of Zn atoms, the number of observed fragments decreases, and starting from three Zn atoms b-type fragment ions are not revealed in the spectrum. Summarizing the results, we can see that, for the case of five Zn atoms, the $[c_9+5Zn]$ and $[y_9+5Zn]$ ions are observed. It indicates that all five Zn atoms are either localized in the fragment KPQQFFG, or most likely that zinc binds to different sites and migrates during fragmentation.

We studied the temperature dependence of the formation of complexes with zinc for different charge states of Substance P ions (Figure S1, Online Supplementary Materials). It can be seen that a triply charged ion readily forms a complex with one zinc

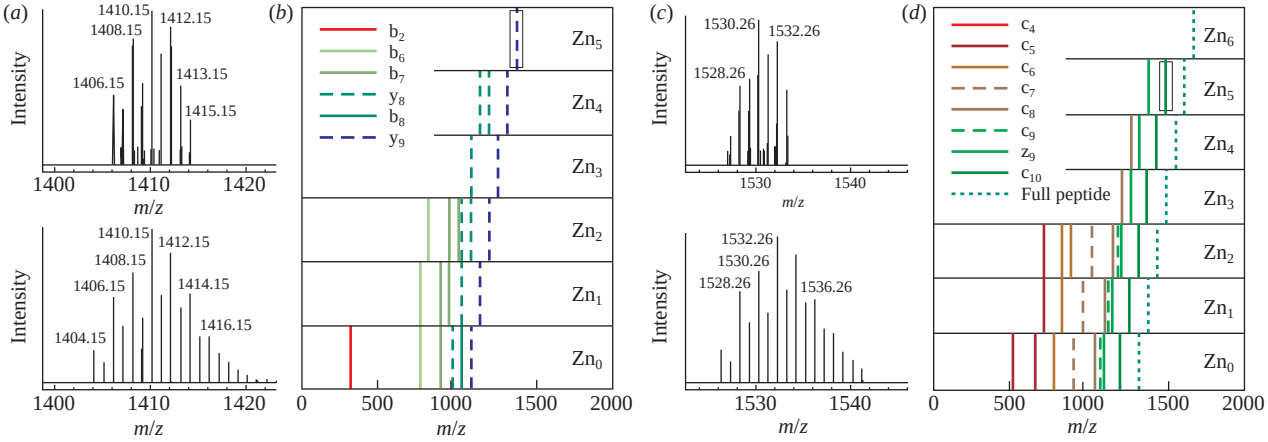


Figure 2 Measured (top) and simulated (bottom) isotopic distribution for the (*a*) y_9 and (*c*) c_{10} fragments of the complex $[M + 5Zn - 8H]^{2+}$. Identified fragments in (*b*) CID fragmentation and (*d*) ECD fragmentation for complexes with different numbers of Zn.

atom at 100 °C. With increasing the temperature, the relative intensity of Zn-adduct decreases and triply charged complexes are not detected at 400 °C. For triply charged peptide, we did not observe complexes with more than one Zn atom. Doubly and singly charged peptides incorporate up to six Zn atoms with the increase of the temperature. Such a behavior may be similar to the supercharging phenomenon that also takes place under specific ESI parameters.[32,33] Different behaviors of different charged states of Substance P in the formation of complexes with Zn are similar to the behavior of different peptides and proteins during gas phase H/D exchange experiments. Lower charge states correspond to more folded conformation and higher to more open. Coulomb's repulsion resulting in starching of the molecule for higher charge states may be a concurrent process to the formation of zinc frame. As a consequence, high charge states will incorporate fewer Zn atoms.

Thus, we found that even the peptide that does not bear Hys or Cys residues can be supermetallized during ESI ionization.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2016.03.008.

References

1 Y. Miller, B. Ma and R. Nussinov, *Coord. Chem. Rev*., 2012, **256**, 2245.
2 H. Sigel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385.
3 D. D. Carlton and K. A. Schug, *Anal. Chim. Acta*, 2011, **686**, 19.
4 G. Drochioiu, M. Manea, M. Dragusanu, M. Murariu, E. S. Dragan, B. A. Petre, G. Mezo and M. Przybylski, *Biophys. Chem*., 2009, **144**, 9.
5 I. Kheterpal, S. Zhou, K. D. Cook and R. Wetzel, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 13597.
6 M. Kraus, M. Bienert and E. Krause, *Rapid Commun. Mass Spectrom*., 2003, **17**, 222.
7 M. I. Indeykina, I. A. Popov, S. A. Kozin, A. S. Kononikhin, O. N. Kharybin, P. O. Tsvetkov, A. A. Makarov and E. N. Nikolaev, *Anal. Chem.*, 2011, **83**, 3205.
8 S. A. Kozin, Y. V. Mezentsev, A. A. Kulikova, M. I. Indeykina, A. V. Golovin, A. S. Ivanov, P. O. Tsvetkov and A. A. Makarov, *Mol. Biosyst.*, 2011, **7**, 1053.
9 P. Faller, C. Hureau, P. Dorlet, P. Hellwig, Y. Coppel, F. Collin and B. Alies, *Coord. Chem. Rev.*, 2012, **256**, 2381.
10 R. N. Grewal, H. El Aribi, J. C. Smith, C. F. Rodriquez, A. C. Hopkinson and K. W. M. Siu, *Int. J. Mass Spectrom*., 2002, **219**, 89.
11 H. Mattapalli, W. B. Monteith, C. S. Burns and A. S. Danell, *J. Am. Soc. Mass Spectrom*., 2009, **20**, 2199.
12 Y. Hoppilliard, F. Rogalewicz and G. Ohanessian, *Int. J. Mass Spectrom*., 2001, **204**, 267.
13 F. Rogalewicz, Y. Hoppilliard and G. Ohanessian, *Int. J. Mass Spectrom*., 2003, **227**, 439.
14 P. F. Hu and J. A. Loo, *J. Am. Chem. Soc*., 1995, **117**, 11314.
15 W. A. Tao, D. X. Zhang, E. N. Nikolaev and R. G. Cooks, *J. Am. Chem. Soc*., 2000, **122**, 10598.
16 F. Turecek, *Mass Spectrom. Rev*., 2007, **26**, 563.
17 C. F. Rodriguez, X. Guo, T. Shoeib, A. C. Hopkinson and K. W. M. Siu, *J. Am. Soc. Mass Spectrom*., 2000, **11**, 967.
18 C. F. Rodriguez, R. Fournier, I. K. Chu, A. C. Hopkinson and K. W. M. Siu, *Int. J. Mass Spectrom.*, 1999, **192**, 303.
19 Y. Kostyukevich, A. Kononikhin, I. Popov, M. Indeykina, S. A. Kozin, A. A. Makarov and E. Nikolaev, *J. Mass Spectrom.*, 2015, **50**, 1079.
20 A. Y. Zherebker, D. Airapetyan, A. I. Konstantinov, Y. I. Kostyukevich, A. S. Kononikhin, I. A. Popov, K. V. Zaitsev, E. N. Nikolaev and I. V. Perminova, *Analyst*, 2015, **140**, 4708.
21 Y. Kostyukevich, E. Zhdanova, A. Kononikhin, I. Popov, E. Kukaev and E. Nikolaev, *J. Mass Spectrom.*, 2015, **50**, 899.
22 Y. Kostyukevich, A. Kononikhin, I. Popov, N. Starodubtzeva, S. Pekov, E. Kukaev, M. Indeykina and E. Nikolaev, *Eur. J. Mass Spectrom.*, 2015, **21**, 59.
23 Y. Kostyukevich, A. Kononikhin, I. Popov, A. Spasskiy and E. Nikolaev, *J. Mass Spectrom.*, 2015, **50**, 49.
24 Y. Kostyukevich, A. Kononikhin, I. Popov and E. Nikolaev, *Eur. J. Mass Spectrom.*, 2015, **21**, 109.
25 I. V. Perminova, I. V. Dubinenkov, A. S. Kononikhin, A. I. Konstantinov, A. Y. Zherebker, M. A. Andzhushev, V. A. Lebedev, E. Bulygina, R. M. Holmes, Y. I. Kostyukevich, I. A. Popov and E. N. Nikolaev, *Environ. Sci. Technol.*, 2014, **48**, 7461.
26 Y. Kostyukevich, A. Kononikhin, A. Zherebker, I. Popov, I. Perminova and E. Nikolaev, *Anal. Bioanal. Chem*., 2014, **406**, 6655.
27 Y. Kostyukevich, A. Kononikhin, I. Popov, N. Starodubtseva, E. Kukaev and E. Nikolaev, *Eur. J. Mass Spectrom.*, 2014, **20**, 345.
28 Y. Kostyukevich, A. Kononikhin, I. Popov and E. Nikolaev, *Anal. Chem*., 2014, **86**, 2595.
29 Y. Kostyukevich, A. Kononikhin, I. Popov and E. Nikolaev, *J. Mass Spectrom*., 2014, **49**, 989.
30 Y. Kostyukevich, A. Kononikhin, I. Popov and E. Nikolaev, *Anal. Chem.*, 2013, **85**, 5330.
31 Y. Kostyukevich, A. Kononikhin, I. Popov, O. Kharybin, I. Perminova, A. Konstantinov and E. Nikolaev, *Anal. Chem.*, 2013, **85**, 11007.
32 K. Chingin, N. Xu and H. Chen, *J. Am. Soc. Mass Spectrom*., 2014, **25**, 928.
33 S. M. Miladinović, L. Fornelli, Y. Lu, K. M. Piech, H. H. Girault and Y. O. Tsybin, *Anal. Chem.*, 2012, **84**, 4647.

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