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LETTER

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Controlling Charge States of Peptides through Inductive Electrospray Ionization Mass Spectrometry

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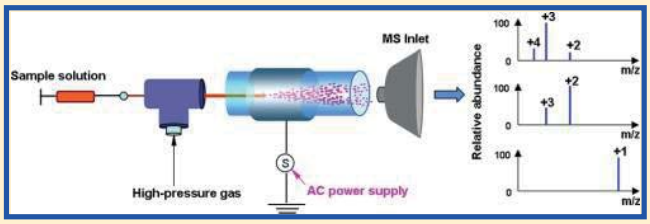
Supporting Information

ABSTRACT: A novel ionization device for controlling the charge states of peptides based on an inductive elecrospray ionization technique was developed. This ion source keeps the major capabilities of electrospray ionization (ESI) which is compatible with liquid separation techniques (such as liquid chromatography (LC) and capillary electrophoresis (CE)) and can be potentially used to control the charge states of peptides accurately by simply varying the AC voltage applied. In comparison with conventional ESI, inductive ESI successfully simplifies the mass spectrum by reducing the charge states of peptide to a singly charged one, as well as eliminating the adduct ions.



Mass spectrometry (MS) has been a powerful tool for proteomics research. Electrospray ionization (ESI)[1] and matrix assisted laser desorption ionization (MALDI)[2] are two typical techniques used to produce gas-phase ions of peptides for their analysis by mass spectrometry. ESI generates multiply charged ions, which facilitate the mass measurement of high mass species by reducing the mass-to-charge (m/z) ratio to a range of values amenable to mass spectrometry.[1] ESI also provides analyte in a continuous homogeneous stream in a repeatable fashion and can be combined with online liquid separation techniques such as high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE).[3] However, the multiplicity of ion charge states for individual species also complicates mass measurement when mixtures of species give rise to ions.[4,5] Compared with ESI, MALDI produces primarily singly charged ions with one major peak on each component on the mass spectrum;[6] thus, it is more suitable for the analysis of mixtures.[7] However, MALDI is difficult to online couple with HPLC or CE. The samples must be purified off-line. MALDI produces ion variation according to the chemical nature and size of the analyte and to the portion of the sample spot selected.[8] In some cases, MALDI desorption is also damaging to the analyte.[9]

Therefore, MALDI-MS is normally used to analyze relatively simple peptide mixtures, whereas integrated liquid chromatography ESI-MS systems (LC-MS) are preferred for analysis of complex peptide mixtures.[10,11] In some case, however, the single-dimension chromatography coupling to ESI-MS may not provide sufficient peak capacity to separate peptide mixtures such as those generated by proteolysis of protein mixture of total cell lysates.[11] In this regard, the development of a new ionization technique that is capable of reducing the charge states of analyte in the ionization process and combining with LC or CE is beneficial to the analysis of complex peptide mixtures.

A variety of methods have been developed to reduce the charge states of biological molecules produced by ESI. Often, this is achieved by changing the solution condition by adding different solvents.[12−14] Solution conditions that reduce the charge states can also lead to poor ESI performance and destroy the native structure of peptide as well. Methods for the control of the charge states by gas-phase proton transfer via ion/ion[15−19] and ion/molecular[20−22] reactions have also been reported, which eliminates the drawbacks posed due to the addition of external solvents. There are also methods developed to control the charge states via liquid-phase interactions between neutral analyte droplets and the charged electrospray, such as extractive electrospray ionization (EESI)[23] and continuous flow-extractive desorption electrospray ionization (CF-DESI).[24] All these methods mentioned above require the introduction of another reagent and thus complicate the experimental setup.

Herein, we describe a novel simple ambient ionization configuration to control the charge states of peptides, termed as inductive electrospray ionization (inductive ESI). This ion source has two major features in that: (1) it has the same structure as that of conventional ESI, except that no DC voltage was applied, and (2) the charge states of biological molecules can be accurately controlled by simply changing the applied AC voltage.

This ionization device consists of two major parts (Figure 1): a conventional ESI emitter without any voltage applied in front of the inlet of the mass spectrometry with an angle of 180°; a quartz tube houses the spray tip. To generate ions, an AC voltage is applied to the tinfoil sheet attached to the outer wall of the quartz tube. Once the AC power supply is turned on, the high electrical

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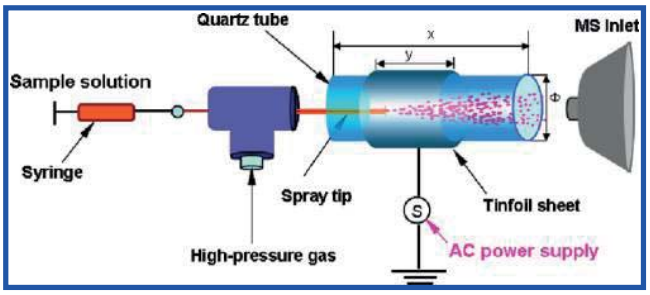

Figure 1. Structure diagram of the inductive ESI apparatus, which consists of a quartz tube containing the ESI emitter. AC voltage is applied on the tinfoil sheet affixed on the outside wall of the quartz tube to charge the analyte and control their charge states. The length of the quartz tube is 50 mm, and the inner and outer diameters of the quartz tube are 5 mm and 7 mm, respectively. The tinfoil sheet is 25 mm long (y).

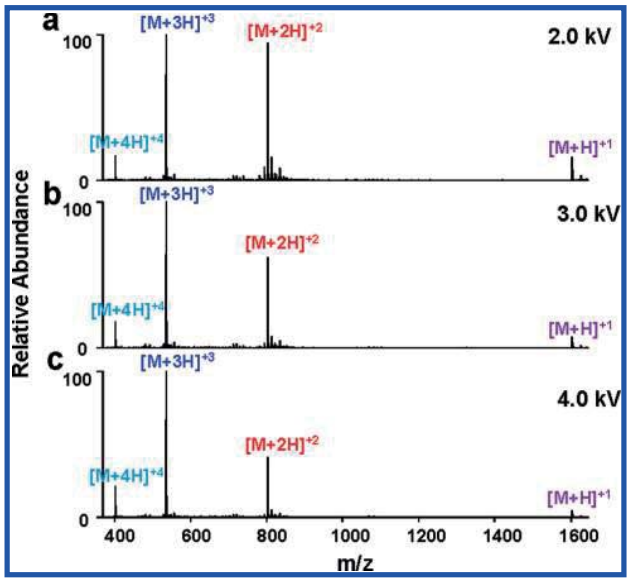

Figure 2. Mass spectra of dynorphin A (1-13) acetate (dissolved in 2:8 (v/v) water/methanol, 1% acetic acid) produced by conventional ESI with DC voltage of (a) 2.0 kV, (b) 3.0 kV, and (c) 4.0 kV.

field produced by electromagnetic induction inside quartz tube induces the generation of charges droplets similar to the previous publication.[25] At a fixed frequency in our present study, the electric field inside the quartz tube would be changing with time rapidly. Therefore, ions passing through the tube would be experiencing an oscillating process, and the amplitudes were dependent on the AC voltage. Multiply charged peptide ions would have a higher amplitude and, therefore, a higher opportunity of charge decay (to peptide ions of lower charge states) due to their impacting the inner wall or colliding with other molecules before they enter the MS. However, the exact mechanism is still unclear, and more research work is needed to elucidate it.

Experiments were carried out using a Thermo LTQ mass spectrometer (Thermo Scientific, San Jose CA): capillary temperature of 275 ° C; capillary voltage of 9 V; tube lens voltage of 100 V. For inductive ESI experiments, the AC potential was applied using an AC voltage supply (CTP-2000K, Suman, China). Applied voltage and frequency were detected using a

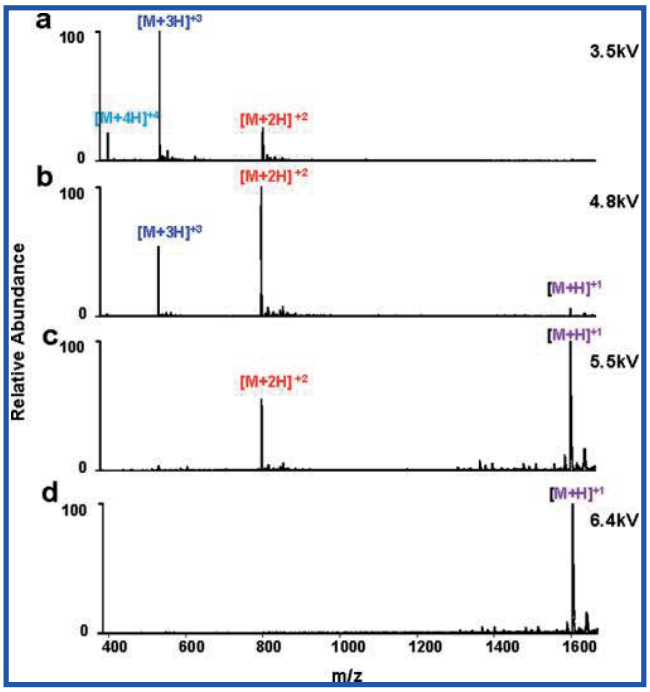

Figure 3. Mass spectra of dynorphin A (1-13) acetate (dissolved in 2:8 (v/v) water/methanol, 1% acetic acid) produced by inductive ESI at (a) 3.5 kV and 8.1 kHz, (b) 4.8 kV and 8.1 kHz, (c) 5.5 kV and 8.3 kHz, and (d) 6.4 kV and 8.5 kHz.

digital oscilloscope (DS1052E, Rigol, China). ESI conditions were as follows: nitrogen gas of 100 psi; the inner and outer quartz capillaries with i.d. 50 μm and i.d. 250 μm, respectively, served as the spray emitter. Sample solution was introduced to the ion source with a flow rate of 2 μL/min. Conventional ESI experiment was performed by removing the quartz tube housed the spray tip. A DC voltage was applied to the needle of syringe in which the sample was loaded.

A series of positive ion mass spectra were obtained in the analysis of peptide dynorphin A (1-13) acetate (MW: 1604) by conventional ESI and inductive ESI. The peptide in the mass spectrum acquired by conventional ESI has four charge states (+1 to +4), and the most abundant peak was the one of +3 charge state. When the DC voltage was changed, there was almost no change on charge state distribution of dynorphin A (1-13) acetate (Figure 2). However, using inductive ESI, the charge state distribution of the peptide was varied obviously by changing the applied voltage (Figure 3). When the peak-to-peak voltage ($V_{p\text{-}p}$) increased from 3.5 kV to 4.8 kV, the charge state distribution was varied from +2 to +4 (Figure 3a) to +1 to +3 (Figure 3b). With a continuous increase of $V_{p\text{-}p}$, the most abundant peak changed from the one of +3 charge state to the one of +1 charge state (Figure 3c). When the $V_{p\text{-}p}$ was increased to 6.4 kV, there was only one +1 charge state peak in the mass spectrum (Figure 3d). Besides the voltage, the length of the electrode (tinfoil sheet) placed on the wall of the quartz tube can also affect the charge states of peptide. Using the same applied voltage, the new ion source with a relative longer tinfoil sheet produced ions with relative lower charge states in our experiments.

The result of charge states controlling the analysis of a simple peptides mixture by the inductive ESI was shown in Figure 4. A

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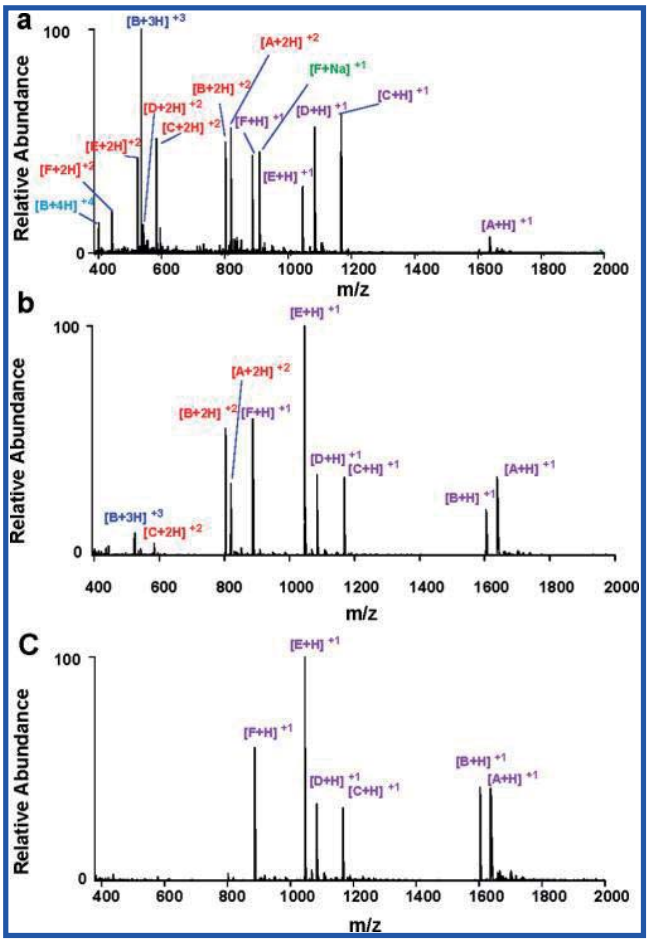

Figure 4. Mass spectra of peptides mixture produced by (a) conventional ESI, (b) inductive ESI (5.1 kV and 8.2 kHz), and (c) inductive ESI (6.5 kV and 8.5 kHz).

Table 1. Detailed Information for Six Peptides A, B, C, D, E, and F

product number	product name	molecular weight	CAS no.
A	somatostatin	1638	38916-34-6
B	dynorphin A (1-13) acetate	1604	72957-38-1
C	alarelin acetate	1167	79561-22-1
D	argipressin acetate	1084	113-79-1
E	angiotensin II	1046	4474-91-3
F	hexarelin acetate	887	140703-51-1

mixture of six peptides were prepared and analyzed by both conventional ESI and inductive ESI. The detailed information of the six peptides was listed in Table 1. The ESI mass spectrum of such a mixture was complex, containing 13 peaks, which correspond to various charge states of the peptides, as is shown in Figure 4a. In contrast, the inductive ESI mass spectrum (Figure 4b) of the same mixture samples had much fewer peaks. When the $V_{p\text{-}p}$ was 6.5 kV, the number of peaks was reduced to only six, each of which was of singly charged state (+1) (Figure 4c). This result demonstrated the reduction of spectral complexity in mixture analysis benefited from charge controlling.

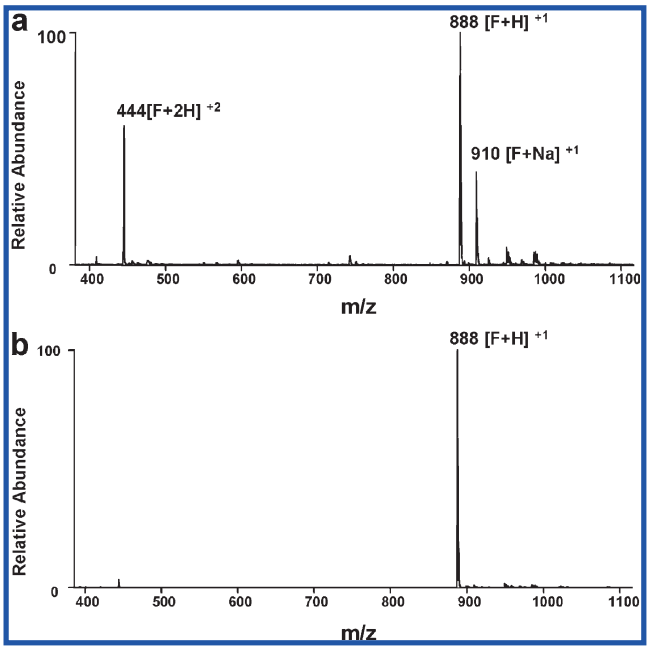

Figure 5. Mass spectra of peptide F produced by (a) conventional ESI and (b) inductive ESI.

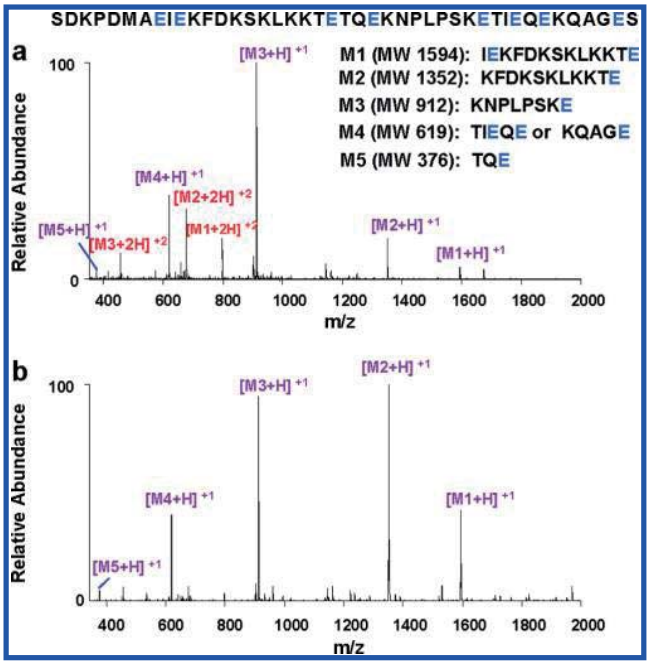

Figure 6. Mass spectra of digested thymosin $\beta 4$ acetate (MW 4963, 38 μM) (a) conventional ESI and (b) inductive ESI (3.7 kV and 8.4kHz).

In addition, singly charged ions are more prone to form adduct ions than multiply charged ions during the charges reduction process, and these adduct ions would compromise mass analysis.[17] The inductive ESI showed the advantage of eliminating adduct ions during the ionization process. This can be verified by the fact that the peak of sodium adducts $[F + Na]^+$ that appeared in the ESI mass spectrum (Figure 4a) disappeared in the inductive ESI mass spectrum (Figure 4b). The single peptide

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F solution was also analyzed by both ESI and inductive ESI. The peaks of both multiply charged ions and $[F + Na]^+$ appeared in ESI spectrum, whereas only one peak of single charged analyte emerged in an inductive ESI spectrum (Figure 5).

We investigated the performance of inductive ESI coupled to mass spectrometry for practical analysis of peptide mixtures produced by enzymatic digestion of polypeptides by endoproteiniase Glu-C. Proteolytic digestion is a common method of generating peptide mixtures for "bottom-up" protemics studies. Glu-C cleaves proteins specifically at the C-terminus of glutamic acid residues in ammonium bicarbonate. The thymosin β4 acetate (200 μg) was digested with 5 μg of endoproteinase Glu-C for 18 h at 37 ° C in 200 μL of 100 mM NH_4HCO_3 (pH 7.8). Then, 10 μL of TFA (10%, v/v) and 840 μL of methanol were added into the mixture. The amino acid sequence of thymosin β4 acetate was listed in Figure 6a (top). After digestion, five peptide fragments were identified by both conventional ESI and inductive ESI. Conventional ESI generated a relatively complex mass spectrum with peaks corresponding to several different charge states of peptides (Figure 6a), whereas inductive ESI generated a simpler mass spectrum with less peaks that correspond to five singly charged peptides (Figure 6b).

In summary, we have proposed a novel ion source termed inductive ESI in the present study. In comparison with conventional ESI, the new ambient ionization method produced variable mass spectra by controlling the charge states of peptide. A simple mass spectrum could be obtained by the reduction of peptide charge states, as well as elimination of the adduct ions. Moreover, it keeps the major advantages of ESI, which is gentle and compatible with liquid separation techniques (HPLC or CE). Therefore, the inductive ESI can potentially be applied in the complex peptide mixture analysis and from which the field of proteomics may benefit.

■ ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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