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On-line Separation and Detection of Peptides by Capillary Electrophoresis / Electrospray FT-ICR-MS

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Abstract: The coupling of capillary electrophoresis to electrospray Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) was presented for the on-line separation and detection of mixture of peptides. With the ultra-high resolution and unmatchable mass measurement accuracy FT-ICR-MS provides, the separation and identification of angiotensin III, octreotide and elastin chemotactic were successfully achieved using this fabricated device, which showed its great application potential in detection and structural identification for multicomponent sample.

Key words: Capillary electrophoresis, FT-ICR-MS, peptide.

Recently, capillary electrophoresis/electrospray ionization mass spectrometry (CE/ESI-MS) has been rapidly developed as a powerful analytical tool for charged species ranged from small molecules such as carboxylic acids[1], phenolic compounds[2], metal species[3], tetramines[4], herbicides[5], drugs and drug metabolites[6] to peptides and proteins[7, 8]. The ESI mode has proven to be sensitive, versatile and relatively easy to use in combination with CE. CE confers rapid analysis and efficient separation and MS provides structural information of analyte.

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is the most powerful MS technology because of its ultra-high resolution and unmatchable mass measurement accuracy. However, up to now there is no commercial CE-FT-ICR-MS instrument. In this paper, we developed a CE-ESI-FT-ICR-MS system for the separation and determination of mixture. Three kinds of peptides, angiotensin III, octreotide and elastin chemotactic, were separated and detected successfully, which showed the great application potential of the equipment in separation and structural identification for multicomponent samples.

Experimental

All experiments were performed on a home-built CE/ESI-FT-ICR-MS system in which both APEX II FT-ICR mass spectrometer (Bruker Daltonics, Inc.) and Spellman high voltage DC supply were used. The overall length of electrophoresis capillary (o.d. 150

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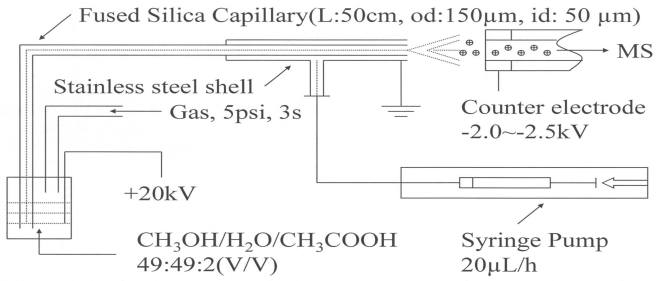
μm, i.d. 50μm) was about 50 cm. The CE capillary was conditioned daily by rinsing with A mixed solvent of CH_3OH: H_2O: CH_3COOH = 49:49:2(v/v), and before each injection the capillary was washed for 5 minutes. Samples were injected by applying a constant pressure of 3.5 kPa for 3 s. During experiment, the CE voltage used was 20 kV, and the potential of electrospray was set at 2.0-2.5 kV. Data acquisition and calculation were performed using software xmass. All the ions were detected in the positive mode.

The homemade CE/ESI interface was shown in **Figure 1**. Both capillary ends were kept at approximately the same level. The sheath liquid made up of the solvent (CH_3OH: H_2O: CH_3COOH = 49:49:2, v/v) was delivered to the probe tip at a rate of 30 μL/h by a syringe pump. No sheath gas or auxiliary gas was used.

Angiotensin III (Arg-Val-Tyr-Ile-His-Pro-Phe, $C_{46}H_{66}O_9N_{12}$, MH^+: 931.5148) and elastin chemotactic (Val-Gly-Val-Ala-Pro-Gly, $C_{22}H_{38}O_7N_6$, MH^+: 499.2874) were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. The octreotide (D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr , $C_{49}H_{66}N_{10}O_{10}S_2$, MH^+: 1019.4477) was synthesized by Meilian Company of XiAn. The three peptides were diluted in triply-distilled water to form 10 pmol/μL solutions and then were mixed together. All the other reagents used in this experiment were of analytical grade.

Figure 1 The configuration of CE-ESI interface

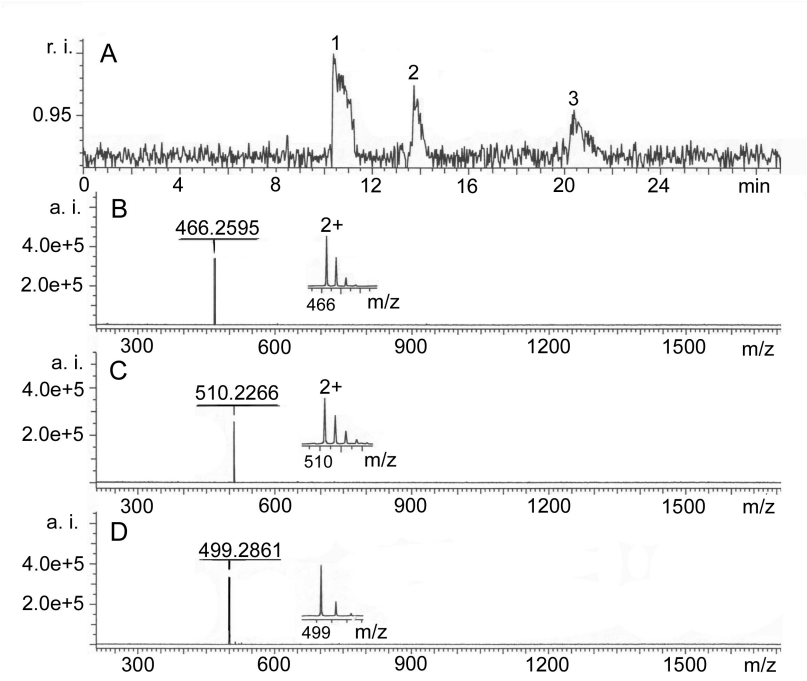


Results and Discussion

The total ion chromatogram (TIC) of the mixture of angiotensin III, octreotide and elastin chemotactic was presented in **Figure 2A**. It was shown that the three peptide components were completely separated from each other in 23 minutes, and that the migration times of angiotensin III, octreotide and elastin chemotactic were 10.5, 14 and 20.5 min, respectively (**Table 1**).

In general, CE separation was controlled by the ratio of charge to mass of species. The larger the charge-mass ratio of species, the faster the electrophoretic rate of species, and the earlier the species eluted. For the peptide mixture separated in this experiment their electrophortic identities was mainly affected by their difference of the basicity. The more the basicity of the peptide, the larger of the charge of peptide and the earlier

Figure 2 TIC chromatogram (A) and the mass spectra of angiotensin III (B), octreotide (C) and elastin chemotactic (D)



the peptide eluted. From the structures of three peptides it can be seen that there are two basic amino acid residues of arginine and histidine in angiotensin III. Especially the arginine residue is at the N-terminal of angiotensin III, which is beneficial for its ability of capturing protons, so the basicity of angiotensin III is the strongest. But in octreotide, there is only one basic amino acid residue of lysine, and the lysine residue is encircled in the ring formed by the two amino acid residues of cysteine, so the ability of capturing protons of the amino acid residue of lysine is greatly suppressed and the basicity of octreotide is reduced. For elastin chemotactic, there is no basic amino acid residue in the sequence. So it could be concluded that the basicity of angiotensin III is the strongest among the three peptides, while the basicity of octreotide is not as strong as that of angiotensin III but stronger than that of elastin chemotactic. Therefore, in our experiments, angiotensin III was eluted at first and octreotide eluted in the second place, while ealstin chemotactic eluted last.

The extracted mass spectra for the three peptides were also presented in **Figure 2**. The doubly protonated ion of angiotensin III (*m/z* 466.2595) and octreotide (*m/z* 510.2266) and the singly protonated ion of elastin chemotactic (*m/z* 499.2861) were clearly shown in **Figure 2B**, **2C**, **2D**. The signal to noise ratio of all mass peaks were larger than 1000. With the highly mass measurement accuracy of FT-ICR-MS (error < 4×10^{-6}), it was easy to calculate the elemental compositions of the three peptides. The experimental and calculated results were summarized in **Table 1**. It can be seen that the

actual detecting results of angiotensin III, octreotide and elastin chemotactic were exactly in agreement with their calculated ones. This advantage is especially useful for the identification of unknown sample. Therefore, it can be concluded from the experimental result that this set of device can be successfully used for the separation and detection of peptide mixtures.

The coupling of modified CE device to FT-ICR-MS offers a novel and efficient sample separation and detection method. The advantages of the CE/ESI-FT-ICR-MS system can be summarized as follows:

1. Low sample consumption was required. In conventional nanospray experiments, 1 μL of sample solution is required at least, while using this modified device, only 20 nL of such solution was needed.

2. Peptide digest can be directly detected without previous separation by using on-line separation and detection device. The peptides mixture can be conveniently, rapidly and easily detected by MS, which will benefit the study of proteomics.

3. Compared to other mass spectrometers, the resolution and mass measurement accuracy of FT-ICR-MS are much higher than other kinds of mass spectrometers. The accurate mass measurements obtained by FT-ICR-MS will facilitate peptide identification using the calculated elemental compositions. Therefore, CE/ESI-FT-ICR-MS can be used for separation and detection of mixtures of unknown peptides.

4. It is convenient to perform on-line tandem MS by FT-ICR-MS. The peptide sequence information sufficient for peptide identification can often be obtained from the product ions resulting from dissociation of the peptide by molecular ion fragmentation. Thus, the structures of components in mixture can be further identified.

Table 1 The experimental and calculated results of three peptides

peptides	t_m (min)	m/z_{exp}	m/z_{cal}	Deviation ($\times 10^{-6}$)
angiotensin III	10.5	931.5117	931.5148	3.3
octreotide	14	1019.4459	1019.4477	1.7
elastin chemotactic	20.5	499.2861	499.2874	2.6

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