Analysis of Peptides by CE/MS

Summary

A synthetic peptide standard containing nine peptides was analyzed by capillary electrophoresis/mass spectrometry (CE/MS).

Experimental

Sample and Buffers

The peptide standard was from Bio-Rad Laboratories (Hercules, CA) and contained the following peptides: bradykinin, angiotensin II, α melanocyte stimulating hormone (α -MSH), thyrotropin releasing hormone (TRH), luteinizing hormone releasing hormone (LHRH), leucine enkephalin, bombesin, methionine enkephalin, and oxytocin. It was prepared in 1 mM acetic acid to give a final concentration of 50 µg/ml for each peptide. Separations were performed using 100 mM formic acid, pH 2.6. Sheath liquid was 70/29.5/0.5 methanol/water/formic acid.

Capillary Electrophoresis

Capillary electrophoresis was performed using the BioFocus® 3000 CE system. The capillary used for the separation was a 50 µm ID x 365 µm OD BioCAP™ linear polyacrylamide (LPA) coated capillary of 75 cm total length. The BioFocus 3000 CE system was interfaced to the Finnigan (San Jose, CA) Atmospheric Pressure Ionization (API) electrospray source using the BioFocus CE/MS interface.

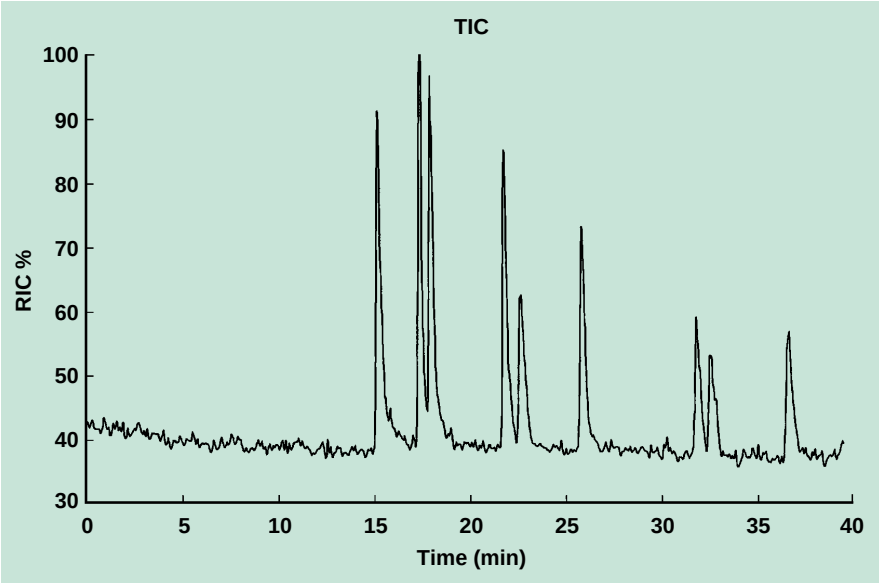

Fig. 1. Total-ion-current electropherogram for the separation of nine synthetic peptides using CE/MS. Conditions: capillary, 50 µm ID x 365 µm OD BioCAP coated capillary with a total length of 75 cm; inlet buffer, 100 mM formic acid, pH 2.6; sheath liquid, 70/29/0.5% methanol/water/formic acid delivered at a flow rate of 3 µl/min; injection, 10 psi*sec; separation voltage, 25 kV; electrospray voltage, +4.5 kV.

Mass Spectrometry

A Finnigan MAT TSQ 700 fitted with a Finnigan API electrospray source was used to perform mass spectrometry. Auxiliary and sheath gas were not used in this experiment. Mass data were collected from 300 to 1,300 mass-to-charge (m/z) units at a rate of 5 sec/scan.

Results and Discussion

Typically, the buffer used for the separation of these peptides is 100 mM phosphate, pH 2.5. Under the phosphate conditions, all nine peptides are baseline resolved. In order to satisfy the requirements of a volatile buffer system for use with CE/MS, 100 mM formic acid was used in this work. The total-ion-current (TIC) electropherogram in Figure 1 reveals that the nine peptides are easily separated with near baseline resolution.

Selected ion plots for the nine peptides are shown in Figure 2. Migration order, charge state, and formula weight for the nine peptides are given in Table 1. The

[1] Foret, F., Thompson, P. V., Karger, B. L., Gebauer, P. and Bocek, P., "Liquid Sheath Effects on the Separation of Proteins in Capillary Electrophoresis/Electrospray Mass Spectrometry," *Anal. Chem.*, 66, 4450-4458 (1994).

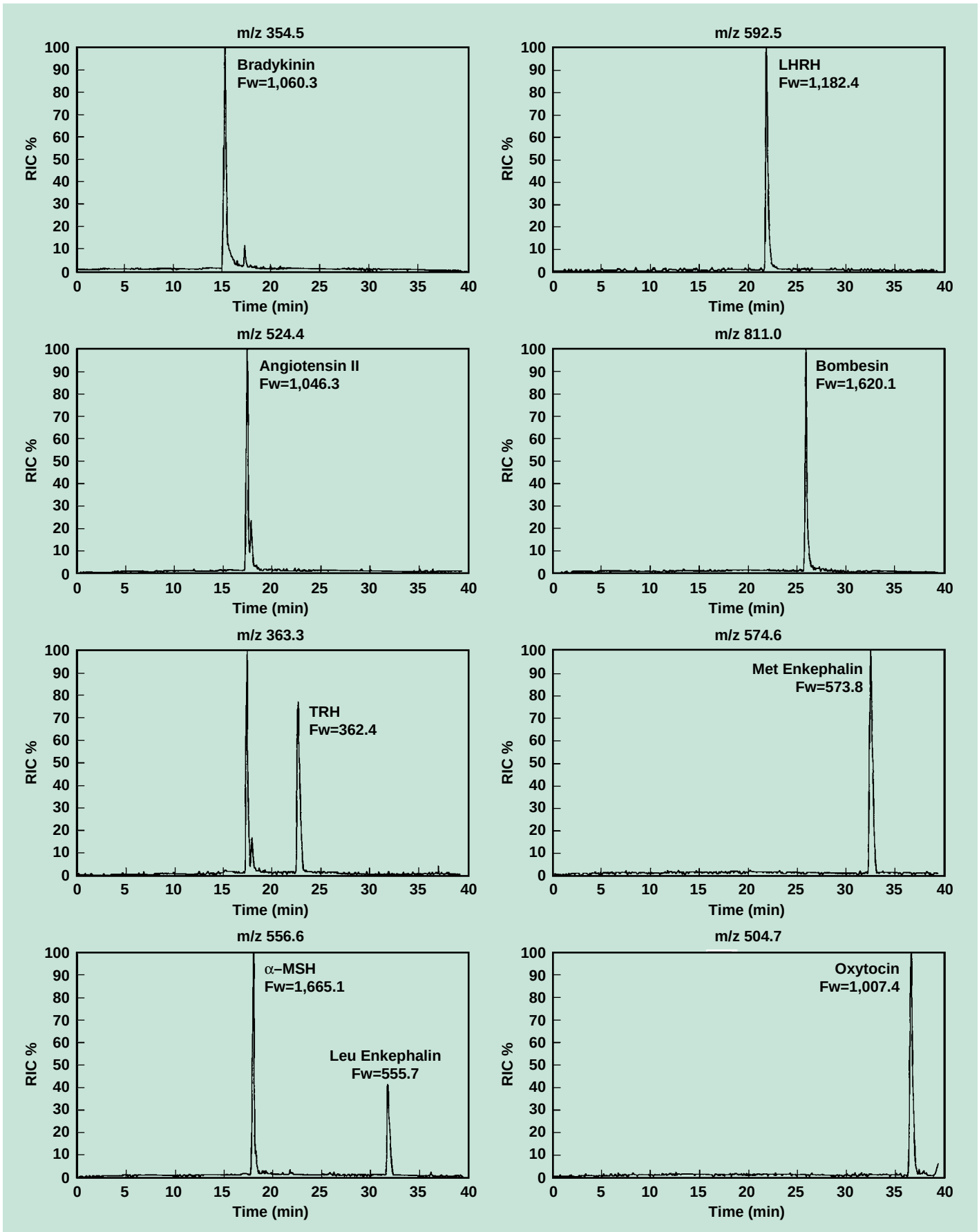

Fig. 2. Selected ion electropherograms for nine peptides. See Figure 1 for conditions.

Table 1. Migration order, formula weight, and charge state of the nine synthetic peptides

Migration Order	Peptide	Formula Weight	Charge State
1	Bradykinin	1,060.3	+3
2	Angiotensin II	1,046.3	+2
3	α-MSH	1,665.1	+3
4	LHRH	1,182.4	+2
5	TRH	362.4	+1
6	Bombesin	1,620.1	+2
7	Leu-Enkephalin	555.7	+1
8	Met-Enkephalin	573.8	+1
9	Oxytocin	1,007.4	+2

first peak in the third selected ion plot (m/z: 363.3) is assumed to be due to fragmentation of angiotensin II.

Under these coated capillary conditions, electroosmotic flow is negligible. This, coupled with the fact that the running buffer and the sheath liquid are of different conductivity, results in an ionic front that moves from the outlet end of the capillary (electrospray side) to the inlet end of the capillary.[1] This front can be either sharp or diffuse depending on the relative conductivities of the running buffer and sheath liquid. The concentration of the formic acid in the sheath liquid was adjusted so that its conductivity was greater than that of the running buffer, but low enough to allow for sensitive mass spectrometric detection. In this way, a diffuse boundary was created between the running buffer and the sheath liquid. Here, as an analyte travels toward the outlet end of the capillary, it migrates through the running buffer, a gradient buffer composed of the original running buffer and that based on the sheath liquid and finally, the new running buffer based on the sheath liquid.

If the conductivity of the sheath liquid is less than that of the running buffer, a sharp ionic front is created. In this case, as an analyte travels toward the outlet end of the capillary, it migrates through the original running buffer and, at some point, goes through the sharp boundary traveling toward the inlet end of the capillary. In this region, the running buffer is based upon conditions derived from the sheath liquid. In this mode, analytes encounter abrupt changes in running buffer which can create changes in migration order, peak efficiency and optical detector artifacts due to abrupt changes in pH, conductivity and run buffer anion. For this reason, the gradual change in pH, conductivity and run buffer anion which accompanies the diffuse front is the preferred running condition for this application.

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Bulletin 1575-54 US/EG (T)96-0152 0596 *SIG 020996 Printed in USA*