

Use of High Performance Electrophoresis in Monitoring Peptide Synthesis

M. Zhu, D. Hansen, S. Burd, V. Huebner,
P. Balasubramanian and A. J. C. Chen
Bio-Rad Laboratories, Richmond, CA 94804

INTRODUCTION

High performance electrophoresis (the HPE™ technique) is a recently developed technique for the separation of biomolecules, using very high voltage in free solution. Separated components are detected in a narrow bore capillary which restricts heat buildup. Because convection and free flow within the capillary are kept to a minimum, high performance electrophoresis can be performed in buffer alone, without agarose or polyacrylamide gel. Electroendosmosis is eliminated because the inner wall of the capillary is coated with a linear polymer. The mobility of the ions (sample or buffer) in a given electric field is strictly dependent upon the ratio of molecular weight and charge. The formula for retention time is

$$C \times \frac{M^{2/3}}{Z}$$

where M = molecular weight, Z = charges at a given pH, and C = constant.

In this study, a standard calibration curve was constructed with data from the fragments of substance P, and this curve was then used to predict the retention time of the nine other peptides.

INSTRUMENTS AND MATERIALS

HPE 100 high performance electrophoresis apparatus from Bio-Rad Laboratories.

Substance P and the fragments SP [1-4], SP [9-11], SP [2-11], SP [8-11], SP [7-11], SP [6-11], and SP [5-11] from Sigma.

Bradykinin, angiotensin II, α -melanocyte stimulating hormone, thyrotropin releasing hormone, luteinizing hormone releasing hormone, [2-5] leucine enkephalin, bombesin, methionine enkephalin, and oxytocin from Sigma.

Peptide products synthesized with the Model 9050 PepSynthesizer™ synthesizer from Milligen.

EXPERIMENTS AND RESULTS

In our first experiment, we characterized the standard calibration curve from substance P and substance P fragments.

Table 1. Amino Acid Sequence of Substance P Fragments

SP Fragments	Sequence	Molecular Weight (M)	Charge (Z)	$\frac{M^{2/3}}{Z}$	Actual Retention Time (Minutes)
SP [1-4]	Arg-Pro-Lys-Pro	496.65	3	20.90	2.50
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH ₂	1347.80	3	40.67	4.15
SP [9-11]	Gly-Leu-Met-NH ₂	318.47	1	46.68	4.65
SP [2-11]	Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH ₂	1191.60	2	50.20	5.25
SP [8-11]	Phe-Gly-Leu-Met-NH ₂	465.66	1	60.08	5.90
SP [7-11]	Phe-Phe-Gly-Leu-Met-NH ₂	612.85	1	72.15	6.70
SP [6-11]	Gln-Phe-Phe-Gly-Leu-Met-NH ₂	741.00	1	81.89	7.25
SP [5-11]	Gln-Gln-Phe-Phe-Gly-Leu-Met-NH ₂	868.15	1	91.00	8.20

Conditions

Sample: Substance P and substance P fragments
 Concentration: 50 µg/ml each component

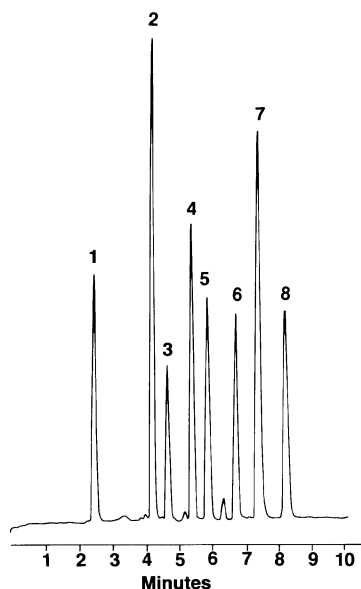
Capillary: 25 µm × 20 cm, coated

Voltage: 8,000 V

Buffer: 0.1 M phosphate, pH 2.5

Detection: UV, 200 nm, 0.02 AUFS

1. Arg-Pro-Lys-Pro
2. Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂
3. Gly-Leu-Met-NH₂
4. Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂
5. Phe-Gly-Leu-Met-NH₂
6. Phe-Phe-Gly-Leu-Met-NH₂
7. Gln-Phe-Phe-Gly-Leu-Met-NH₂
8. Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂



Fragments of substance P

Fig. 1. Standard calibration curve from substance P and substance P fragments.

In our second experiment, we ran nine other peptides using the same conditions as in our first experiment. The retention time and the separation pattern are shown in Figure 2.

Conditions

Sample: Substance P and substance P fragments
 Concentration: 50 µg/ml each component

Capillary: 25 µm × 20 cm, coated

Voltage: 8,000 V

Buffer: 0.1 M phosphate, pH 2.5

Detection: UV, 200 nm, 0.02 AUFS

1. Bradykinin
2. Angiotensin II
3. α-MSH
4. TRH
5. LHRH
6. [2-5] leucine enkephalin
7. Bombesin
8. Methionine enkephalin
9. Oxytocin

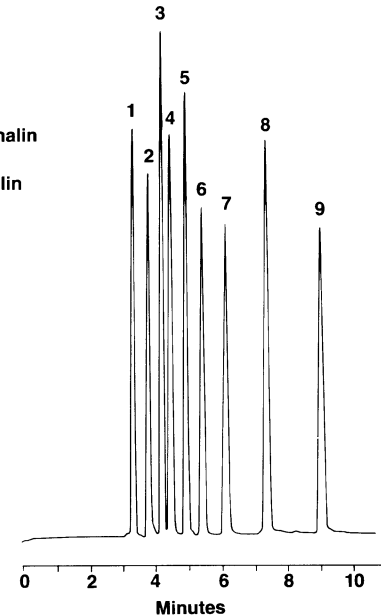


Fig. 2. Electropherogram of nine peptides.

In our third experiment, we generated a substance P standard curve as in our first experiment, and used this curve to analyze the products from a peptide synthesizer.

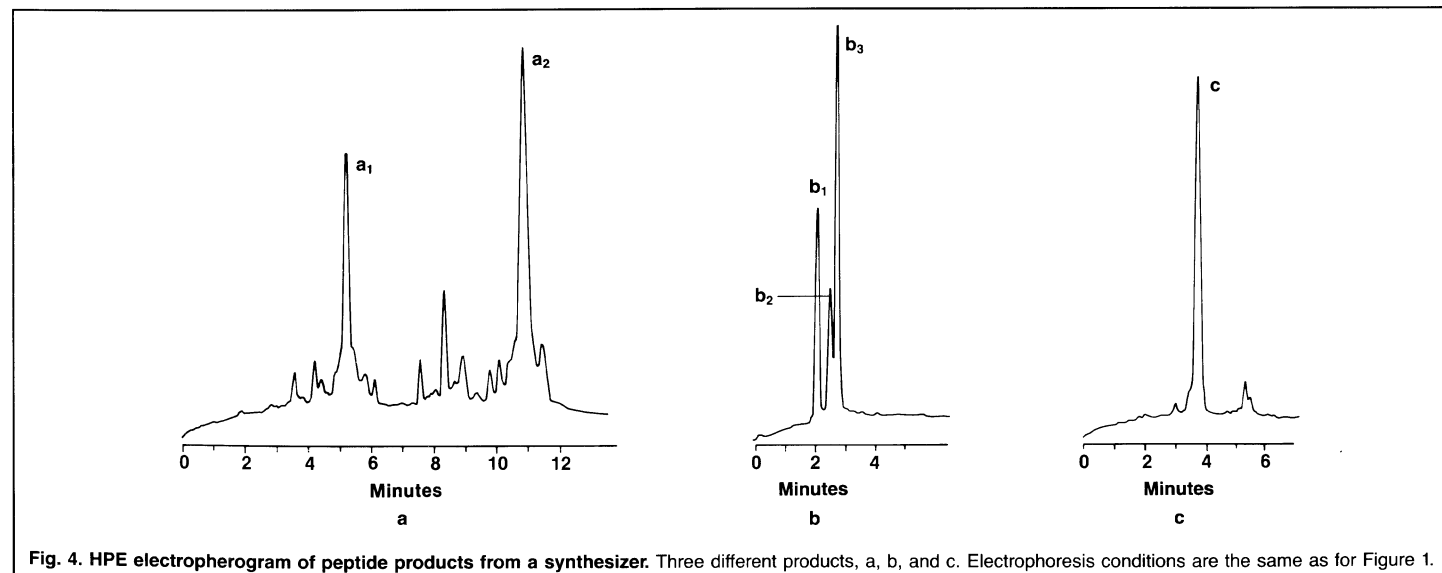
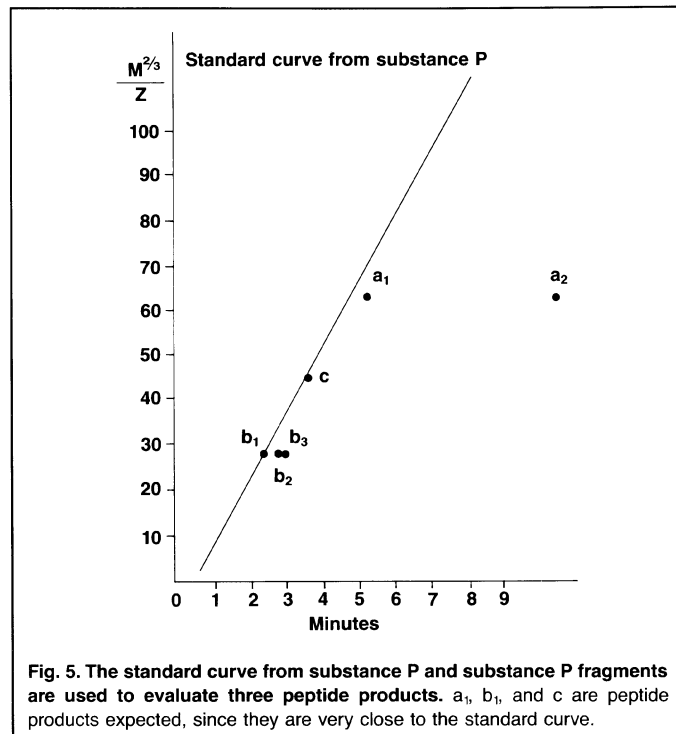
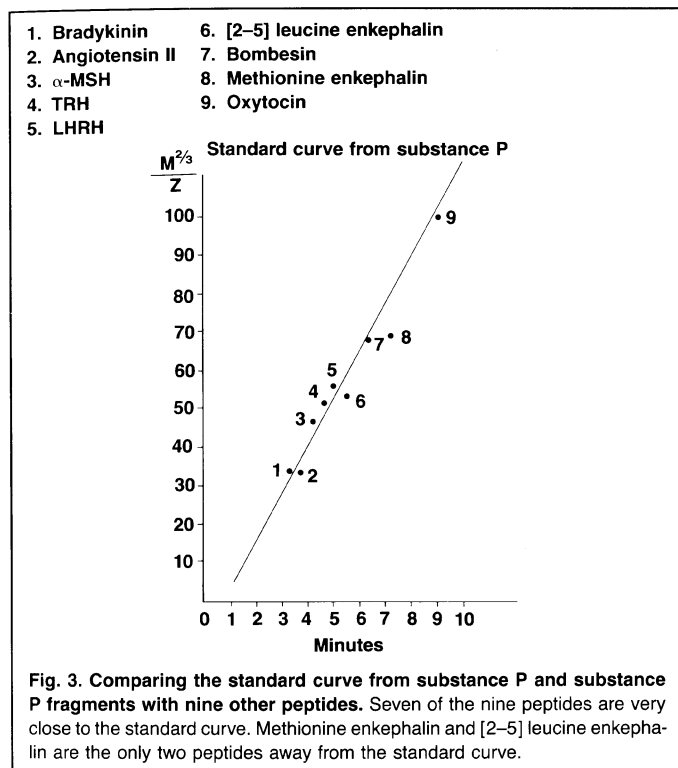
Table 2. Experimental Data for Sample Peptides

Peptide	Sequence	Molecular Weight (M)	Charge (Z)	$M^{2/3}$ Z	Retention Time (Minutes)	
					Expected	Actual
Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH	1060.3	3	34.66	3.52	3.20
Angiotensin II	Asp-Arg-Val-Try-Ile-His-Pro-Phe-OH	1046.3	3	34.35	3.50	3.85
α-Melanocyte stimulating hormone	N-Acetyl-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH ₂	1665.1	3	46.83	4.50	4.20
Thyrotropin releasing hormone	pGlu-His-Pro-NH ₂	362.4	1	50.83	4.82	4.50
Luteinizing hormone releasing hormone	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH ₂	1182.4	2	55.91	5.22	4.95
[2-5] leucine enkephalin	Gly-Gly-Phe-Leu-OH	392.5	1	53.61	5.04	5.40
Bombesin	pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH ₂	1620.1	2	68.97	6.26	6.10
Methionine enkephalin	Try-Gly-Gly-Phe-Met-OH	573.8	1	69.05	6.27	7.25
Oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH ₂	1007.4	1	100.49	8.79	9.00

Table 3. Experimental Data for Crude Synthetic Peptides

Products	Sequence	Molecular Weight (M)	Charge (Z)	Retention Time of Major Components (Minutes)	
				Expected	Actual
a	*Asn-Glu-Asp-Cys*-Gly-Thr-Ser-Gly-Thr-Gln-Gly-Val-Gly-Ser-Pro	1408	2	4.88	a ₁ =5.3, a ₂ =10.7
b	*Pro-Ala-Val-Arg*-Glu-Arg*-Met-Arg*-Arg*-Ala-Gly-Pro-Ala-Ala-Asp	1725	5	2.20	b ₁ =2.1, b ₂ =2.5, b ₃ =2.6
c	*Met-Ala-Gly-Arg*-Ser-Gly-Asp-Ser-Asp-Glu-Asp-Leu-Leu-Lys*-Ala	1565	3	3.55	c=3.6

*Charges at pH 2.5



CONCLUSION

When a polymer coated capillary is used, the electrophoretic mobility of ions depends on the ratio of molecular weight and charge. Using substance P and its fragments for a standard calibration curve, it is

possible to predict the retention time of other peptides if the sequence is known. This method can be used to evaluate the products from a peptide synthesizer.



**Chemical
Division**

Bio-Rad Laboratories Main Office, 1414 Harbour Way South, Richmond, California 94804 • Ph. (415) 232-7000 • Fx. 415 232-4257
Eastern Regional Office, P.O. Box 708, 220 Maple Avenue, Rockville Centre, New York 11571 • Ph. (516) 764-2575 • Fx. 516 764-2186
Also in: Mississauga, Canada • Ph. (416) 624-0713 • Fx. 416 624-3019; North Ryde, Australia • Ph. (008) 224 354 • Fx. 61-2-805-1920;
Vienna, Austria • Ph. (0222) 82 89 01 • Fx. 43-222-828-5629; Brussels, Belgium • Ph. (02) 375.12.40 • Fx. 32-2-374-6162; Paris, France •
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