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Use of High Performance Electrophoresis in Monitoring Peptide Synthesis

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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.

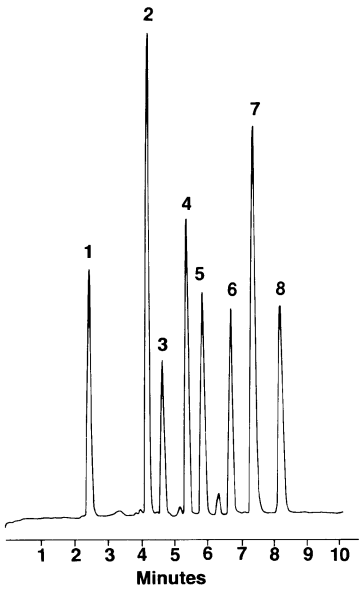
Table 1. Amino Acid Sequence of Substance P Fragments

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-NH_2**
3. **Gly-Leu-Met-NH_2**
4. **Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2**
5. **Phe-Gly-Leu-Met-NH_2**
6. **Phe-Phe-Gly-Leu-Met-NH_2**
7. **Gln-Phe-Phe-Gly-Leu-Met-NH_2**
8. **Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2**



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**

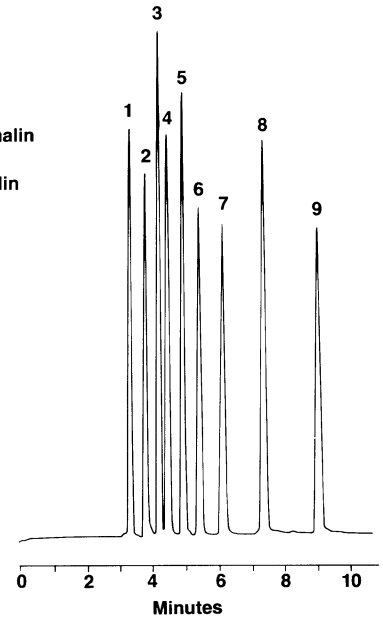


Fig. 2. Electropherogram of nine peptides.

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

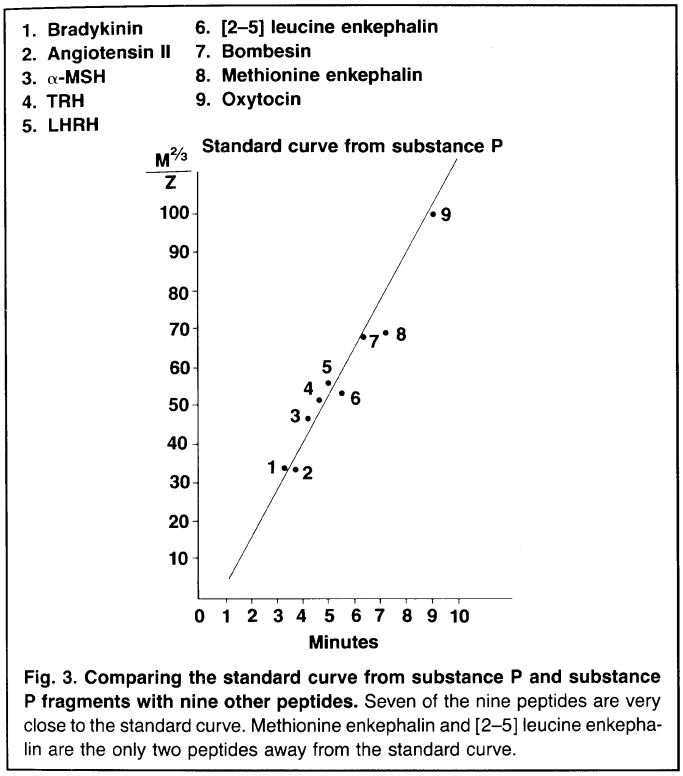


Fig. 3. Comparing the standard curve from substance P and substance P fragments with nine other peptides. Seven of the nine peptides are very close to the standard curve. Methionine enkephalin and [2–5] leucine enkephalin are the only two peptides away from the standard curve.

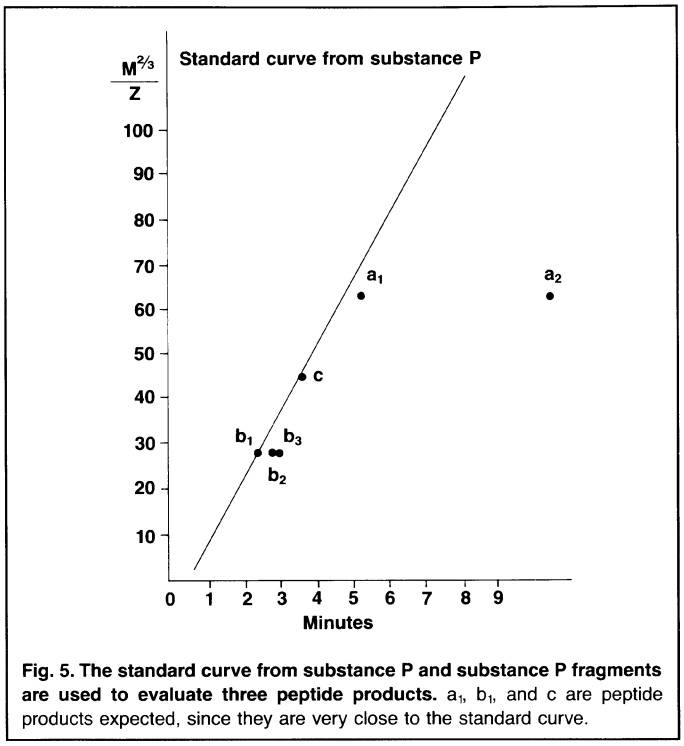


Fig. 5. The standard curve from substance P and substance P fragments are used to evaluate three peptide products. a_1, b_1, and c are peptide products expected, since they are very close to the standard curve.

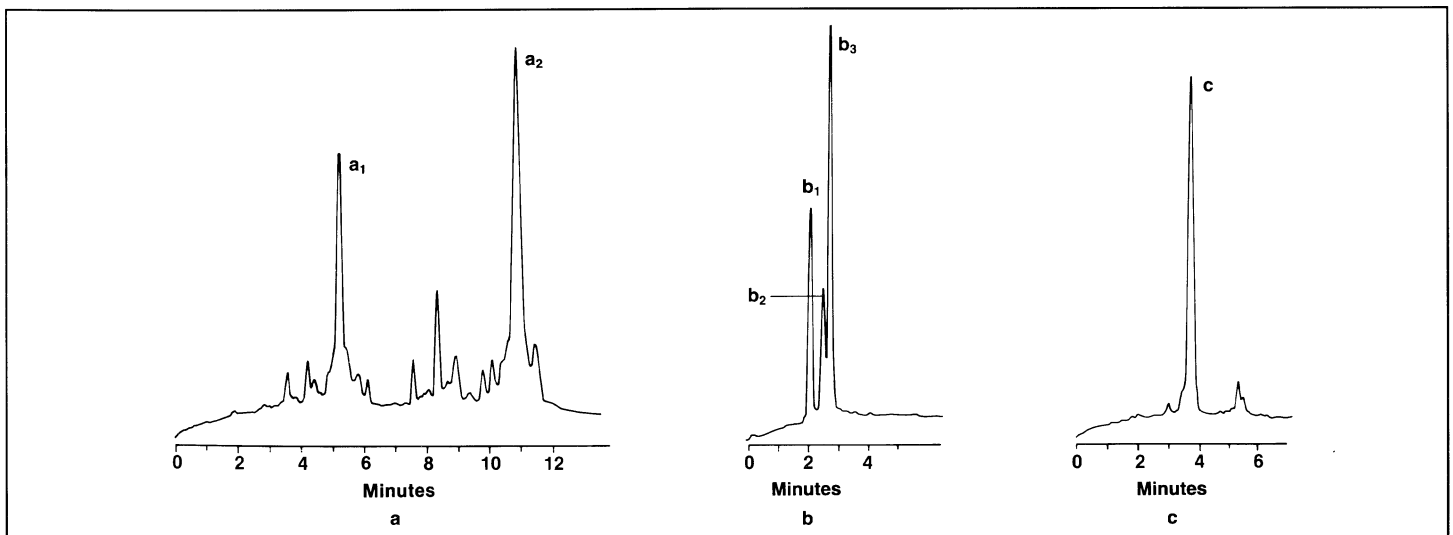


Fig. 4. HPE electropherogram of peptide products from a synthesizer. Three different products, a, b, and c. Electrophoresis conditions are the same as for Figure 1.

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.

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