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US/EG

Bulletin 1480

Use of High Performance Electrophoresis in Peptide Analysis and Peptide Mapping

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8th International Symposium on HPLC of Proteins, Peptides, and Polynucleotides
October 31 – November 2, 1988
Copenhagen, Denmark

INTRODUCTION

Conventional electrophoresis is a widely used method for the separation of many biologically important substances such as proteins and deoxyribonucleic acids. Typically samples are separated by the sieving effect of gels. However, small biological molecules, such as peptides, cannot be effectively separated in this way. The HPE™ technique (High Performance Electrophoresis) can be performed in free solution, i.e., without gel, in such a manner that peptide molecules are separated by their differences in charge-to-mass ratio. In this report, we have explored the use of the HPE technique in peptide analysis and peptide mapping.

The separations take place in polymer coated, fused silica capillary tubes, with significant reduction in electroendosmosis. Peptide molecules differing by deamidation of one amino acid (Figure 3), or differing by substitution of one neutral amino acid (Figure 1), or differing because of the reversal of the sequence of amino acids from end terminal to end terminal (Figure 4). Tryptic digests were all analyzed in the HPE 100 system.

The electrophoretic migration time of the peptides was correlated with a function of the charge-to-mass ratio of the individual peptides (Figure 6). The relationship between the detector response and the sample concentration was also plotted (Figure 5A). The minimum amount of sample loaded into the capillary tube was calculated (Figure 5B).

Isoelectric focusing of three hemoglobin variants was performed in a two-step process in less than 10 minutes from start to finish (Figure 7). Low molecular weight DNA fragments were separated using the sieving effect of a polymer HMC (Figure 8). Impurities in an intentionally aged native human growth hormone were measured using a reverse (negative) polarity in alkaline buffer conditions (Figure 9).

Conditions

Sample:	Neurotensin (2) and neurotensin (guinea pig) (1)
Capillary:	Coated, 25 μm × 20 cm
Voltage:	6.5 kV
Buffer:	0.1 M phosphate, pH 2.56
Detection:	UV, 200 nm, 0.05 AUFS

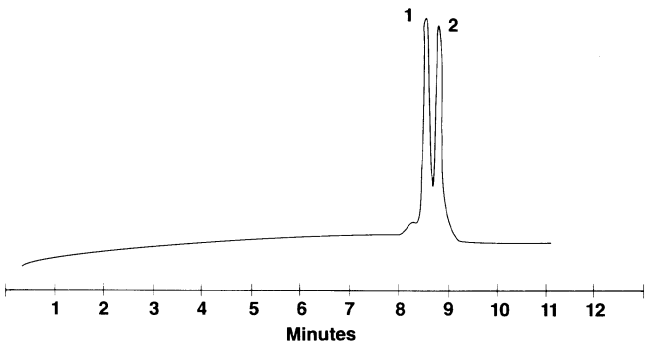


Fig. 1. Neurotensin homologs differing by substitution of one neutral amino acid (serine for proline in the 7 position of the tridecapeptide).

Conditions

Sample: Tryptic digests of BSA
Capillary: Coated, 25 μm × 32 cm
Voltage: 8 kV
Buffer: 0.2 M phosphate, pH 2.56
Detection: UV, 190 nm, 0.02 AUFS

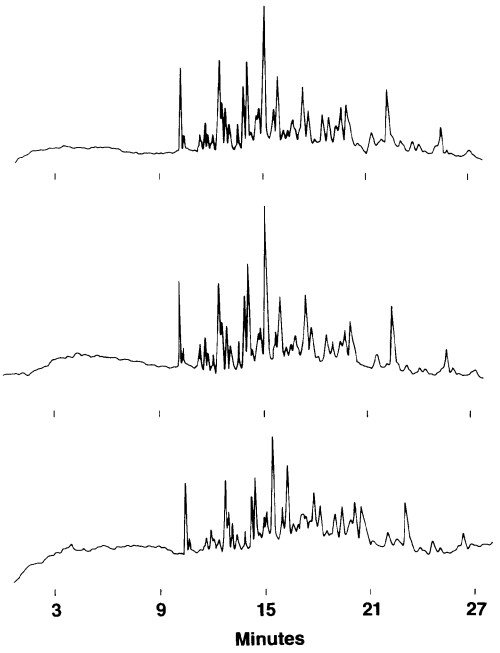


Fig. 2. Repeated tryptic digests of bovine serum albumin followed by HPE analysis, demonstrating total method reproducibility.

Conditions

Sample: Val^5, angiotensin II (2) and Asn^1 Val^5 angiotensin II (1)
Capillary: Coated, 25 μm × 20 cm
Voltage: 8 kV
Buffer: 0.1 phosphate, pH 2.56
Detection: UV, 190 nm, 0.02 AUFS

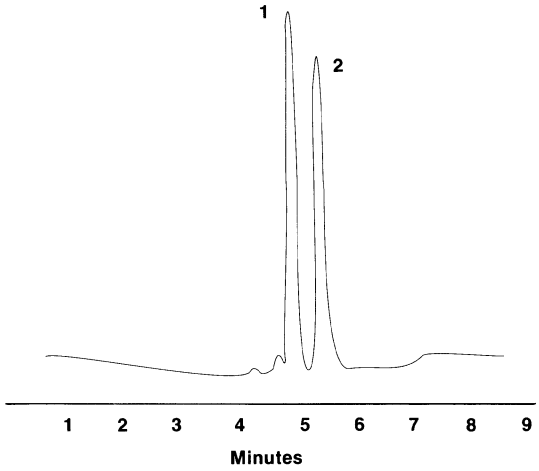


Fig. 3. Peptide degradation. Deamidation of asparagine and glutamine residues in synthetic or natural peptides is a common problem. Since this causes a charge modification, it can easily be observed using the HPE technique.

Conditions

Sample: Gly-Arg-Gly-Asp-Ser (2) and Ser-Asp-Gly-Arg-Gly (1)
Capillary: Coated, 25 μm × 20 cm
Voltage: 6 kV
Buffer: 0.1 M phosphate, pH 2.56
Detection: UV, 190 nm, 0.02 AUFS

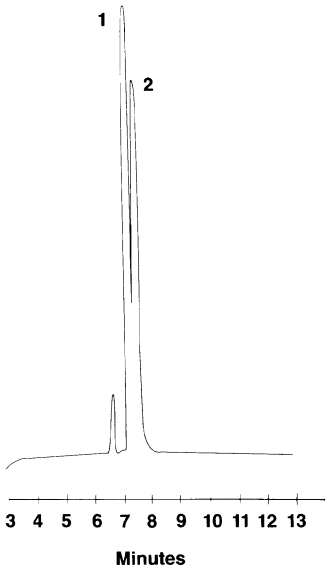
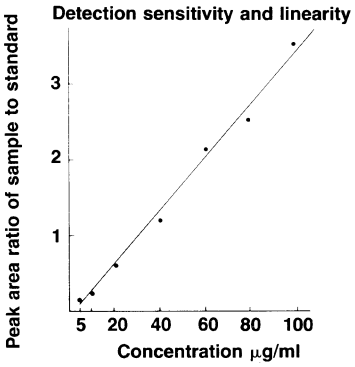


Fig. 4. HPE separation of pentapeptides identical in mass and theoretical charge, differing only by the reverse of the sequence of the same residues.

Conditions

Sample: Human carbonic anhydrase (BSA as internal standard)
Capillary: Coated, 25 μm × 20 cm
Voltage: 8 kV
Buffer: 0.1 M phosphate, pH 2.56
Detection: UV, 200 nm, 0.02 AUFS



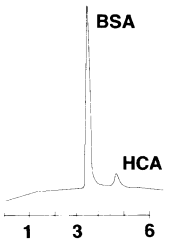
5A.

Sample volume inside capillary:

$$\text{Tube length} \times \frac{\text{sample injection time}}{\text{peak retention time}} \times \text{cross section area} = 2.5 \text{ nl}$$

Sample concentration: 5 μg/ml (pg/nl)

Sample amount inside capillary: 12.5 pg



5B.

Fig. 5. A. Relative peak area vs. sample concentration. B. Amount of human carbonic anhydrate inside capillary.

Conditions

Sample: Substance P fragments 1-4, 1-11, 9-11, 2-11, 8-11, 7-11, 6-11, 5-11
Capillary: Coated, 25 μm × 20 cm
Voltage: 8 kV
Buffer: 0.1 M phosphate, pH 2.56
Detection: UV, 200 nm, 0.02 AUFS

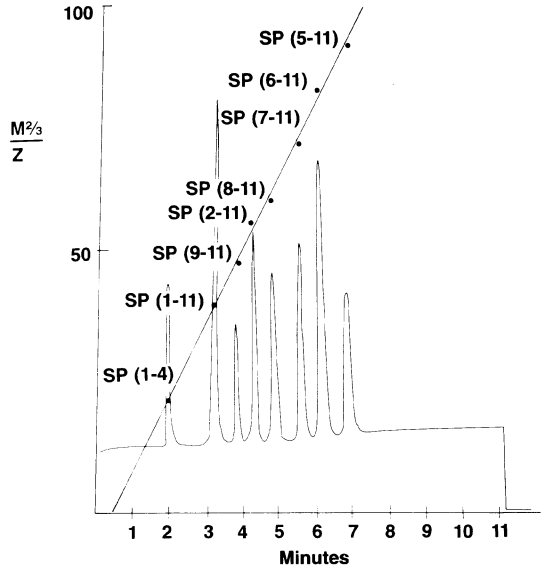


Fig. 6. Standard calibration curve for peptide mobility from Substance P and Substance P fragments.

Conditions

Sample: 0.2 μg/μl DNA size standards
Capillary: Coated, 50 μm × 50 cm
Voltage: 8 kV, negative polarity
Buffer: 0.089 M TBE with 7 M urea, 0.1% SDS, 0.5% HMC
Detection: UV, 260 nm, 0.005 AUFS

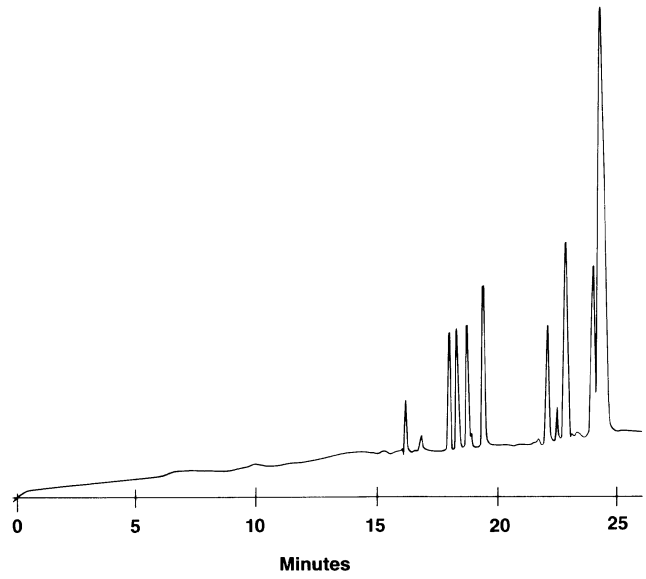


Fig. 8. DNA size standards – low range.

Conditions

Sample: Hemoglobin
Capillary: Coated, 25 μm × 12 cm
Voltage: 8 kV
Anolyte: 20 mM NaOH
Catholyte: 10 mM phosphoric acid
Buffer: 2% Bio-Lyte® ampholyte, 3/10; mobilizer: 20 mM NaOH; 80 mM NaCl
Detection: UV, 280 nm, 0.05 AUFS

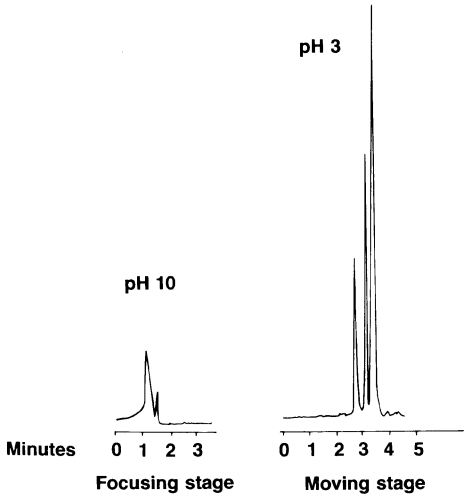


Fig. 7. Hemoglobin – IEF.

Conditions

Sample: Human growth hormone (hGH)
Capillary: Coated, 25 μm × 20 cm
Voltage: 8 kV, negative polarity
Buffer: 50 mM phosphate, pH 8.0; 0.2% HMC; 0.1% G 3707
Detection: UV, 200 nm, 0.06 AUFS

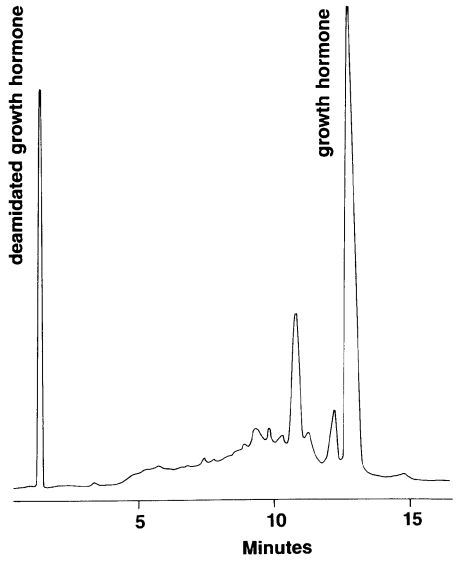


Fig. 9. Human growth hormone.

High performance electrophoresis in free solution has the advantages of being less labor intensive and having much shorter run times compared to conventional electrophoresis. No tedious procedures, such as preparing and staining gels, are required. Run time is generally less than 20 minutes. Samples are easily loaded into capillary tubes electrophoretically with a short pulse of electrical power.

The UV detector on the HPE 100 system has a linear response over a wide range of concentrations. Separated sample zones are detected directly "on-tube", so that no extra column band spreading occurs. Because of the short detection path length, 200 nm wavelength can be used for the detection of peptides. The detector response for peptides at 200 nm is estimated to be 80 times higher than the response at 280 nm. The amount of peptides which can be detected in the capillary is in the picogram range.

Plotting retention time of synthetic substance P fragments with known amino acid sequences against $M^{2/3}/Z$, an equation proposed by Offord[1,2] where M is the molecular weight of a peptide and Z is its valency, a straight line is obtained. This indicates that retention time of peptide may be able to be predicted in a free solution system because the interference of gel is eliminated.

References

1. Offord, R. E., *Nature*, (London) **211**, 591 (1966).
2. Nyberg, F., Zhu, M., Lao, J. L. and Hjertén, S.: High Performance Electrophoresis in Studies of Substance P Degradation, Electrophoresis 88, Copenhagen, 1988.

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