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Comparison of high-performance liquid chromatography and capillary electrophoresis in the analysis of somatostatin analogue peptides

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ABSTRACT

HPLC and CE methods were developed for analysis of somatostatin analogue (S-analogue) peptides utilizing triethylammonium phosphate–organic solvent modifier solvents as the CE buffer and HPLC eluent. Acetonitrile, methanol, ethanol and 2-propanol were applied as organic modifiers. The applicability of HPLC and CE systems was evaluated and compared. Optimum conditions for the separation were determined for both methods. Retention (migration) time, elution order and selectivity can be influenced by modifying the composition of the eluent (buffer) with organic solvents not only in HPLC but also in CE. Although the HPLC system reacted to changes in the organic solvent concentration in a much more sensitive way than the CE system did (from the point of view of retention time), CE proved to be a more suitable method for separating the peptides investigated. Baseline separation could be achieved within 6–9 min by CE, a result which was impossible to achieve with HPLC working in the isocratic mode. In CE the effect of the alcohols on migration times proved to be opposite to that of acetonitrile. Whereas ACN decreased, the alcohols increased the migration times in a concentration-dependent way. The results suggest that CE can be applied very advantageously in peptide analysis. Its performance regarding selectivity, resolution, theoretical plate number, duration and cost is comparable or sometimes superior to that of HPLC.

INTRODUCTION

Capillary electrophoresis (CE) is becoming an increasingly important tool in the analysis of widespread range of molecules. High efficiency, versatility and speed of analysis are among the factors that have promoted the application of the method [1–5].

CE offers many opportunities in analysis, and the information obtainable by CE may complement that which can be obtained by various HPLC methods. Similarly to HPLC, the versatility of the separatory process (which is essential from the point of view of widespread applicability) is very important in CE also [5,6].

There are many parameters that can be used to effect separations and manipulate the selectivity in both CE and in RP-HPLC, including the capillary dimensions, chemical character of the wall of the capillary, buffer composition, ionic strength, pH, applied voltage, sample matrix, buffer additives (*e.g.*, organic solvents, ion-pairing reagents, surfactants) and derivatization in CE [7–19]. In RP-HPLC, separation and selectivity can be influenced by altering the buffer composition, ionic strength, pH, concentration and nature of the ion-pairing reagent, stationary phase and derivatization [20,21].

HPLC is a widely established method in the analysis of peptides, although CE is also of increasing importance for the reasons mentioned

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above [8,9,11,22–24]. As a consequence, the analysis of peptides can be accomplished by either HPLC or CE in many instances.

Comparison of CE and HPLC methods, to decide which is the more advantageous in solving a given analytical task, can be made from several points of view, *e.g.*, resolution, selectivity, sensitivity, peak capacity, duration and cost of the analysis, solvent and chemical consumption of the methods can be compared.

In a previous paper [25] we reported a CE method applicable in the analysis of proprietary somatostatin analogue (S-analogue) peptides (synthesized in our laboratory) utilizing triethylammonium phosphate (TEAP)–organic solvent modifier [acetonitrile (ACN), methanol (MeOH), ethanol (EtOH) and 2-propanol (IPA)] mixtures as buffers. The effect of the organic solvents on the separation process was discussed.

In this work, an HPLC method was applied to the analysis of the same S-analogue peptides, in which the composition of the HPLC eluents was identical with that of the buffers applied in our previous CE analysis [25]. In this paper the results obtained by HPLC are reported together with a comparison of the applicability and advantages of the HPLC and CE systems in the analysis of peptides.

EXPERIMENTAL

Materials

Peptides were synthesized and characterized in our laboratory [25,26]. The structures of the peptides used are as follows:

S-218: D-Phe-Cys-Tyr-D-Trp-Lys-β-Ala-Cys-Thr-NH_2

S-220: D-Phg-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2

S-228: D-Phe-Cys-Tyr-D-Trp-Lys-Pro-Cys-Thr-NH_2

S-232: D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2

S-248: β-Asp(indolinyl)-Cys-Tyr-D-Trp-Lys-Val Cys-Thr-NH_2

S-250: D-Phe-Cys-Tyr-D-Trp-Lys-Leu-Cys-Thr-NH_2

where Phg = phenylglycyl.

Chemicals

HPLC-grade acetonitrile, methanol, ethanol and 2-propanol were purchased from Chemolab (Budapest, Hungary) and orthophosphoric acid and triethylamine from Fluka (Buchs, Switzerland). Water utilized to prepare solutions was treated with an Elgastat UHP water-purification system (Elga, Bucks., UK) to obtain deionized water, free from bacteria and organic contaminants.

Peptide samples at a concentration of 1 mg/ml in water were used both in CE and HPLC analyses.

HPLC analysis

The following conditions were used: flow-rate, 1.2 ml/min (BT 8100 pumps, BT 8300 System Controller; Biotronik, Maintal, Germany); detection, 215 nm; sensitivity, 0.32 on Biotronik BT 8200 UV–Vis detector; sample volume, 25 μl in a 10-μl loop (Rheodyne injector); column, Shandon (Astmoor, UK) ODS-Hypersil, 5 μm (250 × 4 mm I.D.); eluents: 0.083 *M* TEAP buffer (pH 2.25) mixed with different concentrations of ACN or MeOH (15, 20, 25, 30, 35, 40, 45 and 50%, v/v).

Capillary electrophoresis

Capillary electrophoretic analyses were performed with an ISCO (Lincoln, NE, USA) Model 3850 capillary electropherograph with the following conditions: capillary, uncoated silica (45 cm × 50 μm I.D.); detection, 215 nm; sensitivity, 0.02; rise time, 0.8 s; voltage, 30 kV; injection volume, 10 μl through a built-in splitter; splitting ratio, 1:1000; buffers, 0.083 *M* TEAP buffer (pH 2.25) modified with different organic solvents (ACN, MeOH, EtOH and IPA) at different concentrations (0, 5, 10, 15, 20, 25 and 30%, v/v).

Digital data collected from CE runs were stored and analysed with an ISCO Chem-Research controlling and data handling system. The data sampling rate was 8 s^{-1}. Four injections were made for each peptide sample at each buffer or eluent composition. HPLC data were collected and processed with a Biotronik C-R6A Chromatopac integrator with paper speed 1 mm/

min, attenuation 4, width 5, slope 2000 and minimum area 10 000.

RESULTS AND DISCUSSION

Results obtained in the CE analysis of S-analogue peptides [migration times (t_m) *versus* concentration of the organic modifiers in TEAP buffer] have been presented and discussed previously [25]. The migration time, elution order and selectivity can be influenced by modifying the composition of the electrophoretic buffer with organic solvents. Applying different organic solvents as modifiers, the elution order of the peptides can be changed in different ways. At a given concentration the effectiveness of the alcohol depends on its nature: the longer the carbon chain of the alcohol, the greater is the increase in the CE migration time of the peptide compared with that obtained with a buffer not containing any organic solvent. At the low pH applied the peptides migrated mainly owing to their electrophoretic mobility and the electroosmotic component of the net mobility was negligible [7,25].

Table I gives the retention times (t_r) of the S-analogue peptides obtained by HPLC as a function of the concentration of ACN and MeOH in TEAP.

The separation could be influenced in both HPLC (Table I) and CE [25] by changing the organic solvent content of the buffer (eluent).

According to well established knowledge regarding the relationship between t_r and solvent strength, the t_r values in HPLC decreased with increasing organic solvent content of the eluent using both ACN–TEAP and MeOH–TEAP buffers. However, in CE the effect of the alcohols on the t_m values proved to be opposite to that of ACN. Whereas ACN decreased, the alcohols increased the t_m values in a concentration-dependent way [25].

Fig. 1 shows the log t_r and log t_m values of S-218 peptide *versus* the percentage of ACN and MeOH in TEAP buffer. This is a representative plot; similar results were obtained for each peptide with both the ACN–TEAP and MeOH–TEAP buffers.

HPLC is more sensitive than CE to changes in the organic solvent content of the eluent. As Fig. 1 shows, the slopes of the lines for HPLC (solid symbols) are much higher than those of the lines for CE (open symbols) with both the ACN–TEAP and MeOH–TEAP buffers.

Although the t_m values obtained in CE could be manipulated in a less sensitive way than the t_r values obtained in HPLC by changing the composition of the buffers (eluents), CE proved

TABLE I

RETENTION TIMES OF THE S-ANALOGUE PEPTIDES

HPLC analysis performed with ACN–TEAP and MeOH–TEAP eluents (for parameters of the analysis and for eluent compositions, see Experimental). Standard deviations for parallel runs ($n = 4$) are not shown for clarity, but were less than 2%.

Modifier	Concentration (%, v/v)	Retention time (min)					
		S-218	S-220	S-228	S-232	S-248	S-250
ACN	15	13.44	11.70	20.62	13.58	48.52	34.25
	20	5.76	5.74	4.66	4.36	28.20	18.21
	25	2.93	3.21	2.96	2.74	7.13	5.84
	30	2.24	3.02	2.42	2.20	5.00	4.17
	35	2.14	2.19	2.15	2.09	3.51	2.39
MeOH	30	19.40	51.50	15.80	7.89	190.83	113.54
	35	13.01	32.27	11.07	6.40	106.01	55.01
	40	9.19	19.66	7.89	6.15	63.51	36.10
	45	4.78	7.76	4.43	3.81	17.92	12.19
	50	3.61	4.94	3.43	3.13	8.85	7.00

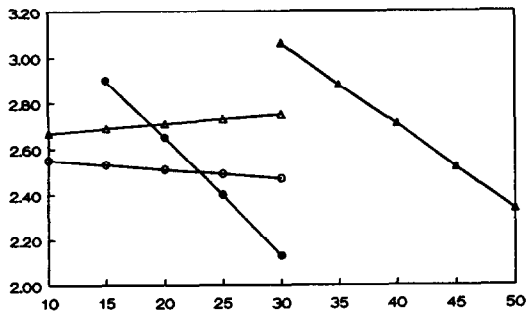

Fig. 1. Log t_r and log t_m values of S-analogue peptide 218 obtained by HPLC and CE analysis *versus* the percentage of ACN or MeOH in TEAP. Abscissa: concentration of ACN or MeOH in TEAP (%, v/v). Ordinate: log t_m and log t_r values. ● = Log t_r obtained by HPLC with ACN–TEAP system; ○ = log t_m obtained by CE with ACN–TEAP system; ▲ = log t_r obtained by HPLC with MeOH–TEAP system; △ = log t_m obtained by CE with MeOH–TEAP system. For data see Table I and ref. 25.

to be a more suitable method for separating the peptides investigated.

Fig. 2 shows the optimized separation of S-analogue peptides by CE with the ACN–TEAP (13.2:86.8, v/v) and MeOH–TEAP (9:91, v/v) systems and by HPLC with the ACN–TEAP (23:77, v/v) and MeOH–TEAP (46.5:53.5, v/v) systems. Optimization was performed by the simplex grid method [27]. Concentration detection limits based on the analyte peak heights and baseline noise level are 5 and 20 μg/ml for HPLC and CE, respectively.

Neither with the ACN–TEAP nor with the MeOH–TEAP buffers is there a direct relationship between the CE migration order and charge/mass ratio of the peptides investigated. For example, the two peptides with the highest charge/mass ratio (5 and 6 in Fig. 2) migrate with the longest migration times with the optimized TEAP–ACN buffer (the sequence of the peptides in decreasing order of charge/mass ratio is S-232 > S-218 > S-220 > S-228 > S-250 > S-248). Interestingly, the other four peptides migrate in decreasing order of their charge/mass ratio with the same buffer. Similarly, there is no direct relationship between the charge/mass ratio and migration order of the peptides with either the pure TEAP or the optimized MeOH–TEAP buffers.

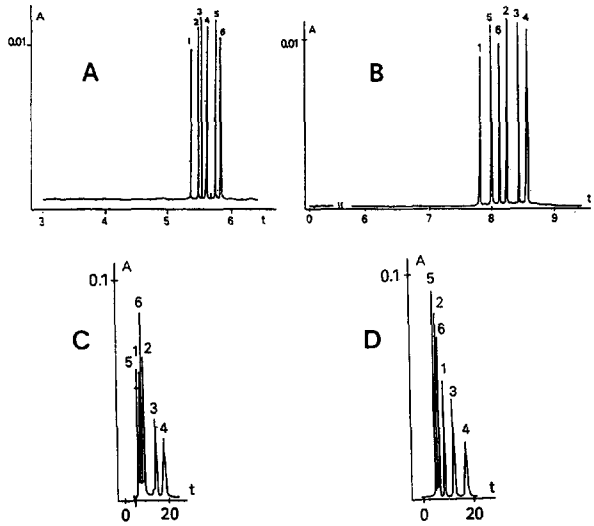

Fig. 2. (A) Electropherogram of the mixture of the six S-analogue peptides obtained with the ACN–TEAP system. Buffer composition: ACN–TEAP (13.2:86.8, v/v). For other parameters, see Experimental. Abscissa: migration time (min). Ordinate: absorbance at 215 nm. Peaks: 1 = S-220; 2 = S-228; 3 = S-250; 4 = S-248; 5 = S-232; 6 = S-218. (B) Electropherogram of the mixture of the six S-analogue peptides obtained with the MeOH–TEAP system. Buffer composition: MeOH–TEAP (9:91, v/v). Other details as in (A). (C) Chromatogram of the mixture of the six S-analogue peptides obtained with the ACN–TEAP system. Eluent composition: ACN–TEAP (23:77, v/v). Other details as in (A). (D) Chromatogram of the mixture of the six S-analogue peptides obtained with the MeOH–TEAP system. Eluent composition: MeOH–TEAP (46.5:53.5, v/v). Other details as in (A).

Baseline separation of the S-analogue peptides could be achieved within 6–9 min by CE, a result which was impossible to achieve by HPLC working in the isocratic mode.

Peptides S-248 and S-250, containing highly hydrophobic moieties [Asp(indolinyl) and Leu moiety, respectively] in addition to the amino acid moieties common in all six peptides, eluted with the ACN–TEAP HPLC system with much higher t_r values than the other four peptides. Baseline separation of the peptide mixture by HPLC could be achieved only by gradient elution of 50 min duration. CE proved not to be sensitive to these hydrophobic moieties of the compounds and similar differences in migration times were not detected.

The net mobility of the analyte in CE is the

sum of the electrophoretic (u_{ep}) and electroosmotic (u_{eo}) mobilities:

$$u_{net} = u_{ep} + u_{eo}$$

$$u_{ep} = e/3 \cdot 10^7 z\eta\sqrt{C}$$

$$u_{eo} = D\zeta_{eo}/4\pi\eta$$

where e is the surface charge density of the analyte, Z the charge of the buffer ion, C the concentration of the buffer ion, η the viscosity of the buffer, D the dielectric constant of the buffer and ζ_{eo} the zeta potential of the capillary wall [7].

Changes in the viscosity and/or the ionic strength of the buffer result in changes in the electrophoretic and electroosmotic mobilities. The alcohols increase whereas ACN decreases the viscosity of the buffer [28,29]. This change in the viscosity could explain the opposite effect of the alcohols and ACN on the net mobilities and therefore on the migration times of the peptides. The higher the viscosity of the alcohol applied, the greater is the increase in the migration time caused at a given concentration.

However, not only the migration times but also the elution order of the peptides can be changed by changing the concentration of the organic solvent [25]. A simple increase or decrease in the viscosity and the ionic strength could only alter proportionately the migration times of each peptide, and could not change their order of elution and their migration times relative to each other.

There is a correlation between the effects of the organic modifiers and their dipole moments (indicating their ability to form associates). ACN decreases the migration times whereas the alcohols increase the migration times (the sequence of the solvents in decreasing order of their dipole moments is ACN > water > MeOH > EtOH > IPA). It cannot be ruled out that the modifiers can interact with the capillary wall and/or can form associates in the buffer.

In CE the effect of the organic solvents cannot be attributed only to the changes in properties of the buffer (viscosity, ionic strength and dipole moment). In addition, organic solvents are able to change the properties of the capillary wall (zeta potential, adsorption–desorption processes between the analyte and the capillary wall) and the properties of the analyte (Stokes radius, mass/charge ratio). These effects may result in changes in the electroosmotic and electrophoretic mobilities of the analyte.

CONCLUSIONS

As in HPLC (where it is a well known and widely applied practice), also in CE the utilization of organic solvents as (eluent) buffer modifiers gives the possibility of manipulating retention (migration) times and selectivity. Utilization of different organic solvents to modify the composition of the electrophoretic buffer in CE even gives the possibility of changing the migration times of the analytes in opposite directions. However, in contrast to HPLC, CE proved not to be sensitive to the hydrophobic moieties of the molecules investigated.

Our results suggest that CE can be applied very advantageously in peptide analysis. Its performance regarding selectivity, resolution, theoretical plate number, duration and cost of the analysis is comparable or sometimes can be superior to that of HPLC.

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