ELSEVIER

Journal of Chromatography A, 680 (1994) 15–24

JOURNAL OF CHROMATOGRAPHY A

Use of zwitterionic detergents for the separation of closely related peptides by capillary electrophoresis

Kimberly F. Greve, Wassim Nashabeh, Barry L. Karger*

Barnett Institute, Northeastern University, 341 Mugar Hall, 360 Huntington Avenue, Boston, MA 02115, USA

Abstract

Capillary electrophoresis incorporating hydrophobic selectivity is shown to be a powerful technique for separating closely related peptide species. In this work, hydrophobic interaction was induced through the addition of suitable amounts of a zwitterionic detergent (N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate) and further modified with organic solvents. A neutral, hydrophilic-coated capillary was used to minimize electroosmotic flow. Two test solutes, Met^{15}- and Leu^{15}-gastrin, were employed to probe hydrophobic selectivity with various electrophoretic conditions. The nature and concentration of the detergent and the organic modifier were varied to adjust the selectivity. Operation near the critical micelle concentration of the zwitterionic detergent in the presence of acetonitrile or various alcohols produced the highest hydrophobic selectivity among the conditions studied. The zwitterionic detergent approach was also briefly compared to the use of non-ionic detergents for hydrophobic selectivity.

1. Introduction

Hydrophobic selectivity is widely used to separate peptides and proteins, e.g. reversed-phase liquid chromatography. Capillary electrophoresis (CE) is a separation technique based on electrophoretic mobility differences among analytes, i.e. charge and mass. The incorporation of hydrophobic selectivity in CE is thus important to broaden the scope of the method for the analysis of peptides and proteins.

Previously, we utilized hydrophobic selectivity in CE for the separation of insulin-like growth factor I (IGF-I) variants through the use of mixed aqueous–organic buffers with various amounts of a zwitterionic detergent [1]. IGF-I is a basic polypeptide composed of 70 amino acid residues with three disulfide bonds. Our work examined five different variants that can be produced in the production of the molecule by recombinant DNA technology. With the modified buffer system, separation of methionine sulfoxide IGF-1 from IGF-1 and IGF-1 from the variant missing the first N-terminal amino acid in its sequence, Gly, was possible. Also, complete identification of all variants was achieved with on-line CE–electrospray mass spectrometry (MS). Since a coated capillary was employed, the neutral zwitterionic buffer modifiers did not enter the mass spectrometer and thus did not provide interference in peak detection or identification.

Traditionally, micellar electrokinetic chromatography (MEKC) [2] has been used for separating neutral, low-molecular-mass compounds by differential partitioning between a micellar phase

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00629-6

and an aqueous buffer phase in CE. The surfactants aggregate to create a pseudo-stationary phase for solute–surfactant interactions *via* hydrophobic, electrostatic, hydrogen bonding, or a combination of these forces. Neutral solutes migrate in order of increasing hydrophobic character, whereas ionic species separate on the basis of a combination of mass-to-charge ratio, hydrophobic character and, in some cases, ion pairing with a charged detergent [3]. Separation of peptides and small proteins that differ in hydrophobicity by MEKC has met with some limited success [4–8].

Most of the above studies used charged surfactants. On the other hand, we have focused on zwitterionic detergents to create hydrophobic selectivity. As with non-ionic detergents, zwitterionic detergents allow pH or ionic strength variation of the running buffer over a wide range of conditions without drastic effects on the properties of the detergent. Secondly, such detergents do not contribute to the solution conductivity, permitting the use of high electric fields without excessive heating of the capillary. Thirdly, these detergents should not, in principle, alter the net charge of the analytes to which they are bound. Finally, these detergents do not, in general, induce biopolymer denaturation.

The purpose of this article is to explore further the zwitterionic detergent approach utilized for the IGF-I variants. Closely related peptides, namely Met^{15}- and Leu^{15}-gastrin; Ala^{1}- and Tyr^{1}-somatostatin; and Met^{13}- and Leu^{13}-motilin were used as test solutes (see Fig. 1 for sequences). The effect of a wide range of operating conditions including the nature and concentration of the organic modifiers as well as the zwitterionic detergent on separation was studied. As previously described, coated capillaries were utilized to minimize electroosmotic flow and thus to be able to determine directly the influence of buffer additives on the electrophoretic mobility of the analytes. It was found that operation near the critical micelle concentration (CMC) of the

Met^{15}-Gastrin I (human) 17 amino acids
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-**Met**-Asp-PheCOONH$_2$
Leu^{15}-Gastrin I (human) 17 amino acids
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-**Leu**-Asp-PheCOONH$_2$

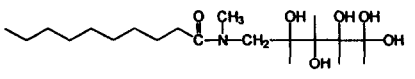
Ala^{1}-Somatostatin 14 amino acids
Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys

Tyr^{1}-Somatostatin 14 amino acids
Tyr-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys

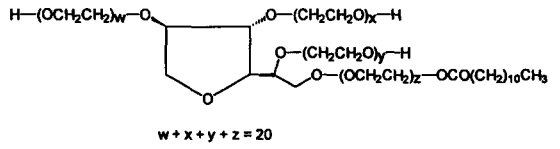
Met^{13}-Motilin (porcine) 22 amino acids
Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-**Met**-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln
Leu^{13}-Motilin (porcine) 22 amino acids
Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-**Leu**-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln

Ile^{5}-Angiotensin II (human) 8 amino acids
Asp-Arg-Val-Tyr-**Ile**-His-Pro-Phe
Val^{5}-Angiotensin II (human) 8 amino acids
Asp-Arg-Val-Tyr-**Val**-His-Pro-Phe

Fig. 1. Peptide sequences.

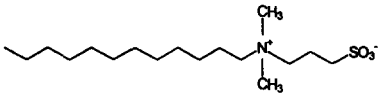


A. MEGA 10



w + x + y + z = 20

B. TWEEN 20



C. DAPS

Fig. 2. Structures of detergents used for this work: (A) MEGA 10 (decanoyl-N-methylglucamide); (B) Tween 20; (C) DAPS (N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate).

zwitterionic detergent with addition of small amounts of organic modifier provided a highly selective separation of the acidic and basic peptides differing by a single neutral amino acid residue. The use of non-ionic detergents at relatively moderate concentrations will also be briefly discussed. Fig. 2 provides the structures of the detergents studied in this work.

2. Experimental

2.1. CE instrumentation and methods

A Beckman P/ACE instrument, version 2100 (Beckman, Palo Alto, CA, USA) was used. System Gold version 7.11 controlled the instrument. The electropherograms were monitored at 214 nm with a data collection rate of 10 Hz. A 75 μm I.D. × 375 μm O.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was coated with a polyvinylmethylsiloxane sublayer and then a layer of polyacrylamide [9]. The capillary, with an effective length of 40 cm and a total length of 47 cm, was kept at a constant temperature of 25°C, and stored in water overnight. A chromatographic software package, Chrom Perfect, was employed to compare and reanalyze electropherograms (Justice Innovations, Palo Alto, CA, USA).

To ensure reproducible separations, the capillary column was purged with fresh buffer for 3 min before each injection. Stock solutions of buffer, detergent, and organic solvent were combined to form the running buffer daily. The pH of the buffer was only adjusted if the organic content was over 20% (v/v).

2.2. Reagents and materials

Samples were obtained from Sigma (St. Louis, MO, USA): Met^{15}- and Leu^{15}-gastrin, Met^{13}- and Leu^{13}-motilin and Ala^{1}- and Tyr^{1}-somatostatin. The detergents, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (DAPS), decanoyl-N-methylglucamide (MEGA 10), and Tween 20 were purchased from Calbiochem (San Diego, CA, USA). Analytical-grade buffer materials [N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (Taps), 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris), ϵ-amino-*n*-caproic acid (ϵACA) and acetic acid] as well as bromophenol blue were purchased from Sigma. HPLC-grade acetonitrile (ACN) and ethanol were obtained from J.T. Baker (Phillipsburg, NJ, USA); methanol, isobutanol and 1-butanol were purchased from Fisher Scientific (Fairlawn, NJ, USA); propanol was obtained from EM Science (Cherry Hill, NJ, USA) and isopropanol from Fluka (Ronkonkoma, NY, USA). Milli-Q water (18.2 MΩ water; Millipore, Bedford, MA, USA) was employed to prepare all buffer and sample solutions. All buffers were filtered with a 0.2-μm pore-size filter (Schleicher and Schuell, Keene, NH, USA) before use.

2.3. CMC determination

CMC measurements were made by determining the absorption of a dye, bromophenol blue (8.3 m*M*), that interacts with the micelle as a function of the concentration of the surfactant [10–12]. The spectra of the solutions were taken

with a Beckman DU-60 spectrophotometer using a wavelength range of 560 to 620 nm and a scan speed of 500 nm/min. The maximum wavelength values were recorded and plotted versus the detergent concentration. The concentration at which a sharp transition occurred in the λ_{max} of bromophenol blue (dye interacting with the detergent) was taken as the CMC.

3. Results and discussion

3.1. Zwitterionic detergent

In order to examine hydrophobic differences between peptides, two test solutes, Met[15]- and Leu[15]-gastrin (Fig. 1), were chosen because of their identical peptide sequence except for the one neutral amino acid substitution. An unsuccessful attempt to separate the peptides using a polyvinylmethylsiloxane–polyacrylamide-coated capillary with a buffer of 20 m*M* Taps–Tris pH 8.0 was made. Since the gastrins did not separate, a system which incorporated an added hydrophobic selectivity was deemed necessary.

The zwitterionic detergent, DAPS (Fig. 2), at concentrations at and above the CMC was chosen for this additional selectivity. At 5 m*M*, Fig. 3A (the CMC is 3.7 m*M* in aqueous solution, see Table 1), some shouldering in the peak as well as broadening and peak tailing was observed. Further increases in the detergent concentration resulted in a single peak being obtained, e.g. 20 m*M* (Fig. 3B). Concentrations up to 50 m*M* DAPS were investigated, and still no separation was observed. Since detergent alone was unsuccessful in separating the species, addition of small amounts of organic modifiers

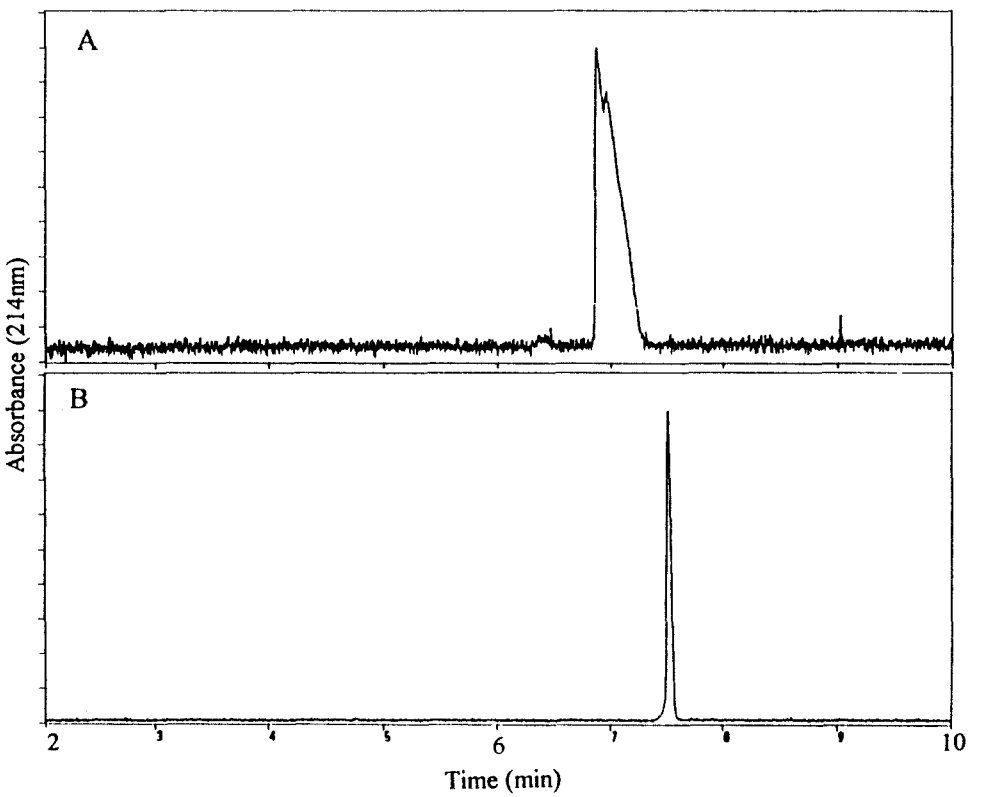


Fig. 3. Separation of Met[15]- and Leu[15]-gastrin with several concentrations of DAPS: (A) 5 m*M*, 9 μA, (B) 20 m*M*, 8 μA; running voltage, 30 kV; 20 m*M* Tris–Taps, pH 8.0. Other conditions are as in the Experimental section.

Table 1
CMC Values for DAPS

Buffer	pH	Organic (%, v/v)	Organic type	CMC (m*M*)
20 m*M* Taps–Tris	8.0	0		3.7
20 m*M* Taps–Tris	8.0	10	ACN	5.0
20 m*M* Taps–Tris	8.0	20	ACN	26.3
20 m*M* Taps–Tris	8.0	30	ACN	60.5
20 m*M* Taps–Tris	8.0	10	Isopropanol	3.5
20 m*M* Taps–Tris	8.0	10	Propanol	3.1
20 m*M* Taps–Tris	8.0	20	Propanol	14.7
20 m*M* β-alanine–citric acid	3.8	2.5	Butanol	2.6

Note: at different pH values the change in CMC was negligible. The CMC in water is 2–4 m*M*.

was tested, based on the results of our previous work [1]. As shown in Fig. 4, the inclusion of 10% (v/v) ACN in the background electrolyte, under otherwise identical conditions to that in Fig. 3A, resulted in baseline separation of the two test solutes. The migration order of the analytes is based on the hydrophobic differences, i.e. the more hydrophobic Leu^{15}-gastrin migrates slower. The analytes presumably interacted differentially with the zwitterionic detergent, and the addition of the small amount of acetonitrile likely affected the extent of that interaction. It is worthwhile to note that the conditions in Fig. 4 are similar as those used to resolve the IGF-I variants [1].

Control experiments were performed to determine if the organic modifier could independently (in the absence of detergent) affect the selectivity of the system. Up to 20% (v/v) ACN was added to the aqueous buffer and still no separation was obtained with the gastrins, although a slight increase in migration time was observed. *n*-Alcohols (methanol through 1-butanol) were also investigated, with no separation observed. Therefore, since the results in Fig. 4 must include the combination of the zwitterionic detergent and organic modifier, the concentration of the detergent as well as the type and amount of the organic modifier were explored further.

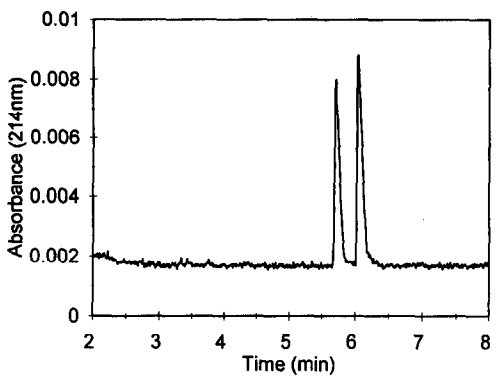


Fig. 4. Complete separation of Met^{15}- and Leu^{15}-gastrin using 10% ACN and 5 m*M* DAPS. All other conditions as in Fig. 3.

With 10% (v/v) ACN, the concentration of hydrophobic selector was varied from 0 to 70 m*M* (Fig. 5). Little or no separation was observed at low and very high DAPS concentrations, but an optimum separation was achieved at moderate concentrations. Relative mobilities, or selectivity, of the two gastrins as a function of DAPS are shown in Fig. 6. A bell-shaped curve is obtained at 10% ACN with an abrupt maximum around the CMC value of DAPS (5 m*M*, see Table 1). Optimum selectivity at 20% ACN was also observed around the CMC (26 m*M*) with a slow reduction in selectivity at higher organic modifier concentrations, ultimately leading to no separation. These plots suggest that the interaction of the analytes with the micelles may be non-selective, because increases in the micelle concentration reduced selectivity. It should be

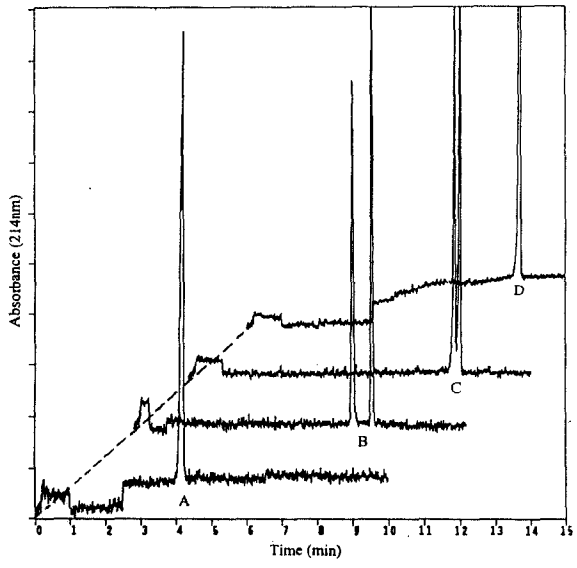


Fig. 5. Electropherograms of the gastrins at constant organic percentage and various DAPS concentrations: (A) 3.5 m*M*, (B) 7.5 m*M*, (C) 20 m*M*, (D) 70 m*M*. All other conditions as in Fig. 3.

noted that at concentrations beyond the CMC, the amount of free detergent remained fixed while the concentration of micelles increased. As discussed previously, it would appear that solute-free zwitterionic detergent interactions are hydrophobically selective. Addition of higher concentrations of DAPS beyond the CMC may simply have increased the relative extent of the non-selective micelle interaction at the expense of the free detergent selective interaction. The lower maximum in the selectivity at 20% (v/v) ACN in comparison to 10% (v/v) ACN and the broad peak may simply reflect weaker hydrophobic interactions with the higher organic composition.

Fig. 7 displays peptide mobility as a function of the detergent concentration at 10 and 20% ACN. With 10% ACN, a sharp change in the mobility is again observed in contrast to 20% ACN. Since the CMC increased upon the addition of organic, more free detergent molecules may be present to interact with the analytes (Table 1: CMC is 5 m*M* with 10% ACN vs. 3.7 m*M* in buffer alone). Furthermore, it appears that the ACN modulates the extent of interaction of DAPS with the peptide to yield optimum selectivity in Fig. 6. At 20% ACN, a gradual decrease in the electrophoretic mobility was obtained with increasing DAPS concentration, eventually approaching the maximum mobility found with 10% ACN. Fig. 7 suggests that the interaction of the analytes with the

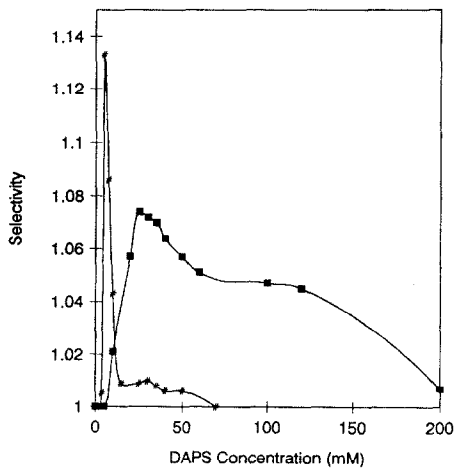


Fig. 6. Effect of detergent concentration at 10% ACN (#) and 20% ACN (■) on selectivity (migration time ratio of Leu^{15}-gastrin/Met^{15}-gastrin). All other conditions as in Fig. 3.

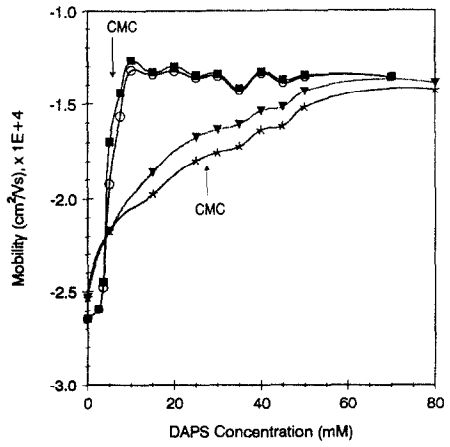


Fig. 7. Effect of detergent concentration at constant organic percentage on the electrophoretic mobility of gastrins. All other conditions as in Fig. 3. ○ = 10% ACN, Met^{15}-gastrin; ■ = 10% ACN, Leu^{15}-gastrin; * = 20% ACN, Met^{15}-gastrin; ▼ = 20% ACN, Leu^{15}-gastrin.

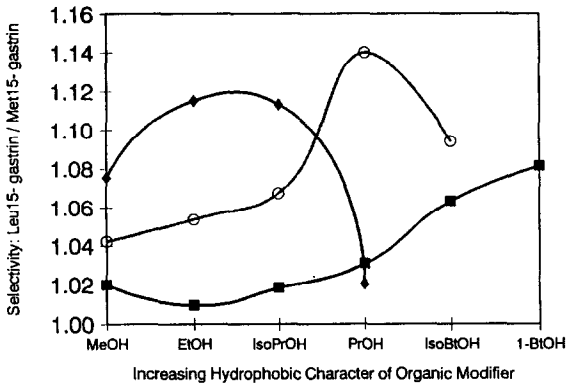


Fig. 8. Effect of organic modifier at constant detergent concentration (■ = 5%; ○ = 10%; ◆ = 20%). Plot of selectivity vs. nature of organic modifier. All other conditions are as in Fig. 3.

micelles is weaker than the free detergent (monomer form).

In our previous report [1], the type of organic modifier was shown to have an effect on the selectivity of the small, closely related IGF-I variants. For example, improved resolution was obtained when the separation of des(Gly)-IGF-I from IGF-I was performed using 1-butanol, relative to ACN. To explore further the role of the organic modifier in manipulating selectivity, CMC measurements of DAPS in the presence of various organic modifiers were first performed. Table 1 shows that ACN disrupts micelle formation regardless of the ACN percentage, as the CMC rapidly increases with concentration of ACN, e.g. 26.3 m*M* for 20% and 60.5 for 30% ACN. Also, low percentages of *n*-alcohols (e.g. propanol and 1-butanol) facilitate micelle formation (i.e. decrease CMC to 3.1 or 2.6 m*M*, respectively) and high percentages (e.g. propanol) reduce micelle formation (i.e. CMC increases to 14.7 m*M*). These results for short-chain alcohols agree with literature findings [13].

We next examined the separation of the gastrins using alcohol modifiers of increasing carbon chain length (i.e. increasing hydrophobic character, methanol through 1-butanol) at a fixed concentration of DAPS, 5 m*M*. In all cases complete separation was achieved near the CMC for each system. Fig. 8 displays a plot of selectivity vs. organic modifier, and Table 2 shows the selectivity vs. the percent of organic modifier at a fixed concentration of DAPS, again 5 m*M*. Increased hydrophobic character (increasing carbon number) of the alcohol shifted the maximum selectivity to lower organic percentages [i.e. 20% (v/v) for ethanol, 12% (v/v) for propanol and 7% (v/v) for isobutanol]. This trend may be related to the reduction of the CMC with increasing chain length of the alcohol (Table 1). At high percentages [greater than 15% (v/v) organic], short-carbon-chain alcohols display bet-

Table 2
Effect of organic modifier at 5 m*M* DAPS

	Selectivity values for Leu[15]-/Met[15]-gastrin								
	Organic (%)								
	2	5	7	10	12	15	20	25	30
ACN	1.01	1.03	1.035	**1.13**	1.037	1.035	1		
MeOH	1.02	1.02	1.05	1.04	1.05	**1.09**	1.08	1.04	1.00
EtOH	1.03	1.01	1.05	1.05	1.06	1.09	**1.12**	1.07	1.02
IsoPrOH	1.02	1.02	1.04	1.07	1.09	**1.12**	**1.11**	1.02	1.01
PrOH	1.02	1.03	1.06	1.14	**1.18**	1.13	1.02		
IsoBtOH	1.03	1.06	**1.13**	1.09					
1-BtOH[a]	1.03	1.08	**1.14**						

Values in bold are maximum values. Bt = Butyl.
[a]1-Butanol will not dissolve at 10%.

ter selectivity than longer-chain alcohols, and at low percentages (less than 7%, v/v) of organic solvent, the opposite is true. As for ACN, the maxima in selectivity occur close to the CMC.

Since the chosen zwitterionic detergents are insensitive to changes in pH, successful hydrophobic-based separation can also be observed at low pH. A pH 4.4 buffer using DAPS was used to separate a mixture of four acidic peptides, Ala^1- and Tyr^1-somatostatin and Met^{13}- and Leu^{13}-motilin (Fig. 1). The somatostatin peptides contain 14 amino acids with a disulfide bond between the third and the last cysteine amino acids, whereas the motilins are 22 amino acids long. Each pair of peptides have similar mass-to-charge ratios and only differ by one neutral, amino acid in their sequence. Separation of the four acidic peptides (Fig. 9) was made possible through the use of 20 m*M* εACA–acetic acid, pH 4.4, modified with 5 m*M* DAPS with either 15% ACN or 5% 1-butanol. The peptides could not be separated without the zwitterionic detergent. Furthermore, the selectivity of the motilins was higher when using 1-butanol as the organic modifier. Fig. 9 demonstrates again that the zwitterionic detergent system with small amounts of organic modifier can be used to resolve peptides that differ in hydrophobicity.

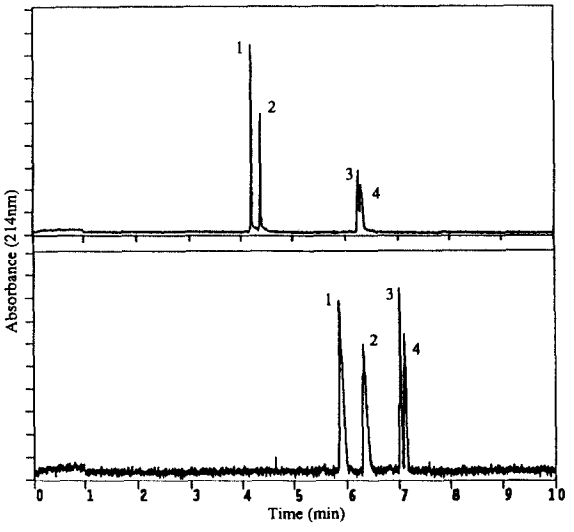


Fig. 9. Separation of Met^{13}- and Leu^{13}-motilin and Ala^1- and Tyr^1-somatostatin. Coated capillary: 37 cm (30 cm effective length) × 75 μm I.D.; running voltage 30 kV; detection at 214 nm; 25°C; buffer, 20 m*M* εACA–acetic acid, pH 4.4 with (top) 15% ACN and 5 m*M* DAPS and (bottom) 5% 1-butanol and 5 m*M* DAPS. Peaks: 1 = Ala^1-somatostatin; 2 = Tyr^1-somatostatin; 3 = Met^{13}-motilin; 4 = Leu^{13}-motilin. See Fig. 1 for amino acid sequence.

3.2. Neutral detergents

Neutral detergents can be used as an alternative to the zwitterionic detergents for hydrophobic selectivity. MEGA 10 (Fig. 1) was previ-

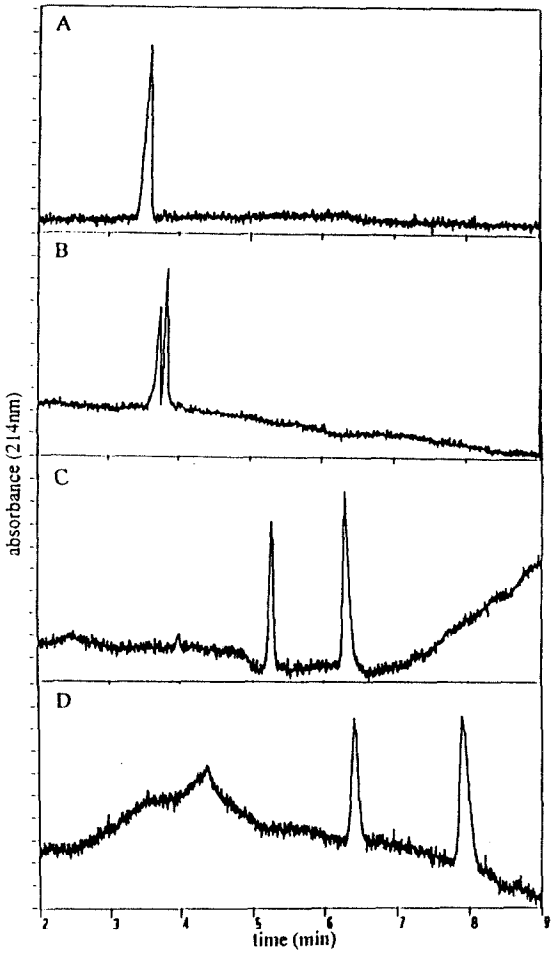


Fig. 10. Separation of Met^{15}- and Leu^{15}-gastrin using MEGA 10. Coated capillary: 47 cm (40 cm effective length) × 75 μm I.D.; running voltage 30 kV; detection at 214 nm; 25°C; buffer, 20 m*M* Taps–Tris pH 8.0 containing various amounts of MEGA 10: (A) 5 m*M*, (B) 10 m*M*, (C) 30 m*M*, (D) 50 m*M*.

ously used in MEKC as an anionic-borate complex for the separation of neutral and charged herbicides [14]. For our purposes, a coated capillary that eliminated electroosmotic flow and reduced analyte adsorption was again utilized in conjunction with MEGA 10 to separate closely related peptides (Fig. 10). The hydrophobic interaction imparted by this neutral detergent allows complete separation of the closely related peptides, Met^{15}- and Leu^{15}-gastrin above the CMC. (The CMC of MEGA 10 is 16.9 m*M* at pH 8.) In contrast to DAPS, increasing the concentration of MEGA 10 above the CMC improves separation, suggesting that the micelle partitioning may be selective. The problem with using MEGA 10 as the surfactant, however, is the unstable baseline at 214 nm due to the UV absorbance of the amide bond in the detergent. In an attempt to lower the amount of detergent needed for the separation, organic modifiers were investigated. This approach was not successful, in that addition of 10% ACN to 30 m*M* MEGA 10 decreased the selectivity of the two analytes, and 10% ACN with 10 m*M* MEGA 10 resulted in no separation.

Another neutral detergent that has been previously used is Tween 20 (Fig. 2), which allowed separation of closely related angiotensin II and motilin peptides using a bare silica capillary [5]. It was found that Tween 20 required quite high concentrations (80–250 m*M*, well above the CMC of 100 μM) to obtain the proper selectivity of peptides. This can be contrasted with the zwitterionic detergent used in this work where ca. 5 m*M* was sufficient for separation.

4. Conclusions

Hydrophobic selectivity is an important separation factor for peptide and protein species. In general, CZE with simple buffer systems is inadequate for the separation of closely related peptides having similar mass-to-charge ratios. Therefore, incorporating a hydrophobic mechanism into CE is important for its application to protein chemistry. Separation of closely related peptides that differ in hydrophobicity can be achieved under mild conditions using low concentrations of organic modifier and zwitterionic detergent, such as DAPS. Optimum selectivity is generally observed in the region of the CMC of DAPS in the presence of organic modifiers. Comparisons of relative mobilities of the gastrins suggest that the interaction of the micelles with the analytes may be non-selective, because increases in the micelle concentration did not improve the selectivity of the analytes. To enable the separation of a wide variety of species, the selectivity can be adjusted by varying the nature and concentration of the organic modifier as well as the concentration of the zwitterionic detergent. Using a coated capillary with no electroosmotic flow enhances reproducibility and makes the system simple to manipulate since additives to the buffer will only affect electrophoretic mobility and not simultaneously influence electroosmotic flow, as found, with bare silica [15]. An alternative approach to the use of zwitterionic detergents with organic modifiers is nonionic detergents depending on the sample and the overall separation desired of the closely related peptide species. Also, organic modifiers and zwitterionic detergents in conjunction with coated capillaries are compatible with on-line electrospray MS, since the detergent does not migrate into the mass spectrometer [1]. A more detailed report of the use of detergents with MS will be published separately [16].

Acknowledgement

The authors gratefully acknowledge support of the NIH under GM15847. Contribution No. 605 from the Barnett Institute.

References

[1] W. Nashabeh, K.F. Greve, D. Kirby, D.H. Reifsnyder, S. Builder, F. Foret and B.L. Karger, *Anal. Chem.*, 66 (1994) 2148.
[2] S. Terabe, K. Otsuda, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.

[3] M.G. Khaledi, in J.P. Landers (Editor), *Handbook of Capillary Electrophoresis*, CRC Press, Boca Raton, FL, 1993, pp. 43–93.
[4] H.K. Kristensen and H. Hansen, *J. Liq. Chromatogr.*, 16 (1993) 2961.
[5] N. Matsubara and S. Terabe, *Chromatographia*, 34 (1992) 493.
[6] M.A. Strege and A.L. Lagu, *Anal. Biochem.*, 210 (1993) 402.
[7] H. Gaus, A.G. Beck-Sichinger and E. Bayer, *Anal. Chem.*, 65 (1993) 1399.
[8] T. Yashima, A. Tsuchiya, O. Morita and S. Terabe, *Anal. Chem.*, 64 (1992) 2981.
[9] D.K. Schmalzing, C.A. Piggee, F. Foret, E. Carrilho and B.L. Karger, *J. Chromatogr A*, 652 (1993) 149.
[10] S. Hjertén, L. Valtcheva, K. Elenbring and D. Eaker, *J. Liq. Chromatogr.*, 12 (1989) 2471.
[11] P. Mukerjee and K.J. Mysels, in *Critical Micelle Concentrations of Aqueous Surfactant Systems; NSRDS-NBS 36*, US Goverment Printing Office, Washington, DC, 1971, pp. 1–21.
[12] B.V. Normand and J. Eiselé, *Anal. Biochem.*, 208 (1993) 241.
[13] W.L. Hinze, in W.L. Hinze and D.L. Armstrong (Editors), *Ordered Media in Chemical Separations*, American Chemical Society, Washington, DC, 1987, pp. 6–9.
[14] J.T. Smith, W. Nashabeh and Z. El Rassi, *Anal. Chem.*, 66 (1994) 1119.
[15] G. Frosberg, G. Palm, A. Ekebacke, S. Josephson and M. Hartmanis, *Biochem. J.*, 271 (1990) 375.
[16] D. Kirby, K.F. Greve, W. Nashabeh, F. Foret, P. Vouros and B.L. Karger, presented at the *42nd ASMS Conference on Mass Spectrometry, Chicago, IL, 29 May–3 June 1994*, poster No. 124.