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Micellar electrokinetic chromatographic study of the interaction between enkephalin peptide analogs and charged micelles

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Abstract

The relative hydrophobicity/hydrophilicity of the pentapeptides leucine enkephalin (LE), methionine enkephalin (ME) and five analogs, differing in their uncharged side chain and/or chirality, was investigated by micellar electrokinetic capillary chromatography (MEKC) employing anionic and cationic surfactants. The effect of sodium dodecyl sulfate (SDS) concentration on peptide mobility was studied at pH 8.8, a value that is well above the peptide isoelectric point, to minimize electrostatic interaction with the anionic micelles. Similarly, the effect of cetyltrimethylammonium bromide (CTAB) cationic micelles on peptide migration was studied at pH 4.1. The migration order from MEKC experiments was compared to the peptide hydrophobicity calculated from reversed-phase HPLC-derived hydrophobicity coefficients. Although relative peptide hydrophobicity was, in general, positively correlated with effective electrophoretic mobility, a tryptophan-containing analog showed only weak interaction with micelles compared to the less hydrophobic peptides. The enkephalins studied were zwitterionic in character from pH 3 to 8, and their migration as a function of pH under MEKC conditions demonstrated that electrostatic forces were at least as important as hydrophobic interactions in pentapeptide–micelle complexation.

Keywords: Leucine enkephalin; Methionine enkephalin; Peptides

1. Introduction

Stereospecific binding of opiates to animal brain homogenates was discovered in the 1970s, leading to the identification of a class of endogenous neuropeptides called enkephalins. Methionine- and leucine-enkephalin (ME, LE) are bioactive pentapeptides that are involved in pain response and that exhibit a specific recognition with opioid receptors. At least four main opioid receptors are known (μ, δ, κ, σ), and enkephalins show binding affinity for the μ- and δ-receptor [1]. Their recognition capacity may or may not be conserved if small structural changes occur [2]; therefore, enkephalins and their analogs have been the subject of many studies. ME and LE analysis in brain tissues has traditionally been carried out by radioimmunoassay (RIA) [3]. Enkephalin identification and quantitation from synthetic mixtures or tissue homogenates has also been performed by liquid chromatography with spectroscopic or amperometric detection [4,5] and by mass spectrometry [6]. More recently, capillary electrophoretic (CE) techniques have been applied to the separation [7–9] and tissue analysis [10,11] of ME, LE and related peptides.

The separation of a complex mixture of peptides was one of the first demonstrations of the power of CE [12], although an electrophoretic mechanism alone cannot often separate peptides that differ by substitution of one or two hydrophobic residues. Therefore, the introduction of micellar electrokinetic chromatography (MEKC) [13], which is a CE-based

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separation of neutral analytes based on a hydrophobic mechanism, was eagerly embraced. MEKC has been widely applied – from separations of chlorinated phenols [14] and derivatized amino acids [15] to pharmaceutical analysis [16]. However, MEKC has not been as widely used for peptide separations or physico-chemical studies of peptides because the zwitterionic nature of peptides leads to relatively non-specific electrostatic interactions with charged micelles, masking hydrophobic effects. For example, eight angiotensin peptide analogs with a net positive charge at pH 7 could not be separated under anionic-MEKC conditions, but were almost completely resolved with cationic micelles [17]. A different set of eleven angiotensin analogs could not be separated in anionic or neutral micelle solutions at pH 7, although resolution was improved at pH 2, but only for neutral micelle MEKC [18]. Separation of oxytocin and seven vasopressin analog peptides was not effected with either anionic or neutral micelles, yet was possible with zwitterionic micelles [19]. Mixtures of enkephalin-related peptides containing two to eight amino acids have been separated by MEKC, using micelles of taurodeoxycholic acid at pH 3.1 in a coated capillary [20]. However, an excellent separation of the same analytes could also be obtained by free-zone CZE at pH 3 [9] because these analytes differed significantly in their charge-to-mass ratios.

Micellar solutions are frequently employed as membrane model systems and have been used to investigate hydrophobic interactions with neuropeptides by UV–Vis spectroscopy and NMR [21,22]. Extrapolation of these studies suggest that MEKC could also be a useful tool to model membrane interactions, in addition to probing structure-dependent binding of peptide analogs. Because peptides are zwitterionic at experimentally useful pH values, hydrophobic, hydrophilic and electrostatic interactions contribute to interaction with charged micelles. The present work investigates the effects of an anionic micelle (SDS), cationic micelle (CTAB) and pH on the MEKC separation of enkephalin-like pentapeptides.

2. Experimental

2.1. Materials

Leucine enkephalin (LE), methionine enkephalin (ME), five enkephalin analogs (amino acid sequences are listed in Table 1), sodium tetraborate, acetone, lauryl sulfate (SDS) and cetyltrimethylammoniumbromide (CTAB) were purchased from Sigma (St. Louis, MO, USA) and used without further purification. Monobasic and dibasic sodium phosphate were from Anachemia (Montreal, Canada). In-laboratory distilled water was passed through a multi-cartridge Millipore water filtration/deionization system before use. A fused-silica capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA). Platinum wire and microcentrifuge tubes (1.5 ml and 600 μl) were obtained from Fisher Scientific (Montreal, Canada). Nylon syringe filters,

Table 1
Name, amino acid sequence, molecular mass (M_r) and hydrophobicity[a] of the enkephalin and enkephalin analog peptides

No.	Peptide name	Sequence	M_r	Hydrophobicity	
				pH 2	pH 7
1	D-Trp^2-methionine enkephalin	Tyr^1-D-trp^2-gly^3-phe^4-met^5	702.9	19	21.3
2	D-Ala^2-leucine enlephalin	Tyr^1-D-ala^2-gly^3-phe^4-leu^5	569.7	14.8	17
3	D-Ala^{2,3}-methionine enkephalin	Tyr^1-D-ala^2-D-ala^3-phe^4-met^5	601.7	14.4	16.4
4	Leucine enkephalin	Tyr^1-gly^2-gly^3-phe^4-leu^5	555.7	12.6	14.6
5	Ala^2-methionine enkephalin	Tyr^1-ala^2-gly^3-phe^4-met^5	587.7	12.2	14
5′	D-Ala^2-methionine enkephalin	Tyr^1-D-ala^2-gly^3-phe^4-met^5	587.7	12.2	14
6	Methionine enkephalin	Tyr^1-gly^2-gly^3-phe^4-met^5	573.7	10	11.6
7	Met(O)^5-enkephalin	Tyr^1-gly^2-gly^3-phe^4-met(O)^5	589.7	<10[b]	<11.6[b]

[a] Hydrophobicity of peptides was calculated using the retention coefficients of Guo et al. [33].
[b] Hydrophobicity estimated.

0.2-μm pore size, were purchased from Chromatographic Specialties (Brockville, Canada).

2.2. Background electrolyte and sample preparation

Stock peptide solutions (1 mg/ml) were prepared in Millipore-purified water and stored at −5°C for up to twenty days. After this time, several by-products appeared; therefore, samples were discarded and remade fresh. Analyte mixtures were diluted to give a final concentration of approximately 1 μ*M* for each peptide. Peptides were identified by spiking the mixtures, leading to controlled variations in the peak heights, as observed in Section 3. Background electrolyte (BGE) buffers were prepared in pure water and passed through a syringe filter before replenishing the CE buffer reservoirs. Buffer pH was adjusted by mixing different amounts of monobasic and dibasic sodium phosphate solutions. Acetone was added to the samples as the electroosmotic flow (EOF) marker.

When switching from SDS to CTAB in the MEKC experiments, the capillary was first washed with several column volumes of HCl and pure water, then conditioned overnight with the new surfactant solution. If this procedure was not followed, severe hysteresis effects led to migration time R.S.D.s of up to 35%. In general, two to four injections were made for each change in experimental parameters (surfactant concentration or pH) and the mean effective mobilities are plotted (EOF-corrected; see Section 3). Errors in effective mobilities ranged from 1.5% to 6% R.S.D.

2.3. Apparatus

All experiments were performed on an in-laboratory built capillary electrophoresis–thermo-optical absorbance detection (CE–TOA) system, described previously [23]. Separations were carried out at room temperature (21±1°C) in a 45 cm×50 μm I.D., 190 μm O.D. fused-silica capillary tube with high voltage applied in the range of +8 to +14 kV for SDS–MEKC or −8 to −14 kV for CTAB–MEKC experiments. Samples were injected hydrodynamically at a height of 6 cm for 30 s, timed with a stopwatch. On-column detection was performed 40 cm from the injection end of the capillary where a window was made by burning off 5 mm of the capillarys polyimide coating.

3. Results and discussion

The five bioactive enkephalin analogs that were chosen for our study differed from methionine (ME) and leucine enkephalin (LE) in their uncharged side chains (Table 1). On the basis of tabulated pK_a values [24], all seven peptides were expected to have the same charge, same isoelectric point (p*I* 5.45) and very similar charge-to-mass (q/M_r) ratios. Although no separation was predicted under free-solution CZE conditions near pH 5.45, 2 to 4 very broad peaks were typically seen. The appearance of more than one peak meant that there was not a unique p*I* and that neutral residues have the power to affect the pK_a of the peptide termini. The influence of neighboring amino acid hydrophobicity on peptide pK_a has been previously reported [25]; however, peptide conformation may also account for these variations. For example, a bend in the peptide backbone could place a hydrophobic side chain spatially close to the N- or C-terminus, inducing a change in the amine or carboxylic acid pK_a. LE is known from X-ray crystallographic studies to be able to adopt a single-bend or double-bend conformation stabilized by two intramolecular hydrogen bonds, whereas ME has only been observed in the fully extended chain form [1]. Conversely, ME was shown by NMR and molecular modelling to adopt a single-turn structure in the presence of 50 m*M* SDS [26]. The N-terminal dissociation constants (pK_{a2}) for ME and LE were determined recently, by microtitration, to be 7.36 and 7.40, respectively [27]. This difference in the pK_{a2} values might be influenced by peptide conformation.

Determining relative peptide hydrophobicity by CE is ideally done by studying MEKC interactions with ionic influences eliminated. Although non-ionic micelles are suited for such an experiment, the mixture of analytes must differ enough in their charge-to-mass ratio otherwise no separation will occur. Unfortunately, negligible interaction of enkephalin-related peptides with micelles was reported for two types of neutral surfactant [9]. Therefore, we decided to use MEKC to probe simultaneously the

hydrophobicity/hydrophilicity of seven related peptides under electrostatic repulsion conditions: anionic micelles (SDS–MEKC) at high pH where peptides are predominantly anionic, and cationic micelles (CTAB–MEKC) at low pH where peptides are predominantly cationic. Fig. 1A and Fig. 1B show typical MEKC electropherograms obtained in the two cases.

3.1. SDS–MEKC

MEKC separations are based on a mixed electrophoretic and chromatographic mechanism. Surfactant is added to the background electrolyte (BGE) at a concentration higher than its critical micellar concentration (cmc), and the micelles that are consequently formed are dynamic structures that can complex an analyte. A charged surfactant possesses an intrinsic electrophoretic mobility in free-solution that is typically oriented against the electroosmotic mobility μ_{eof}. The migration of a neutral analyte is

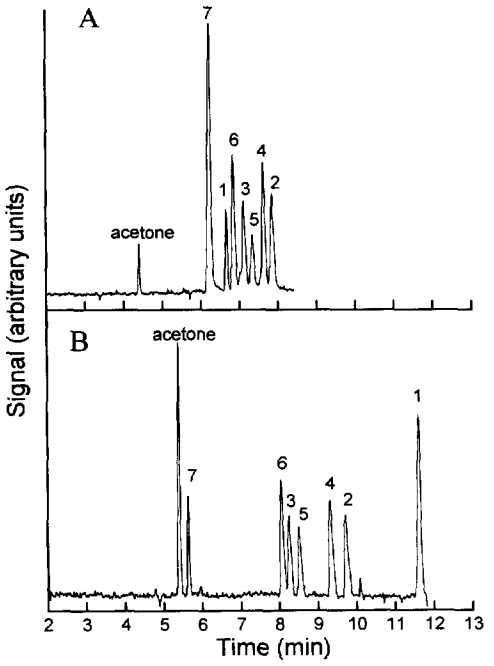

Fig. 1. MEKC electropherograms of a mixture of enkephalins and analogs (see Table 1 for structures) and acetone, the EOF marker. (A) Separation in 50 m*M* SDS, 50 m*M* sodium phosphate, pH 8.8 at +11 kV. (B) Separation in 30 m*M* CTAB, 50 m*M* sodium phosphate, pH 4.1 at −10 kV.

based on its partition, related by the retention factor k, between the aqueous phase and the hydrophobic micellar phase [28]. On the other hand, a charged analyte possesses an intrinsic electrophoretic mobility in aqueous free-solution, μ_{a-aq}, plus the possibility to complex with the micelles through hydrophobic and electrostatic interactions, adding an electrophoretic mobility term based on the analyte–micelle complex, μ_{a-mc}. Therefore, the observed or apparent electrophoretic mobility (μ_{app}) of a charged analyte in MEKC depends on the fraction of time that it spends in free-solution versus complexed with the micellar phase, plus the electroosmotic flow (EOF) mobility coefficient according to Eq. (1) [29,30].

$$\mu_{app} = \left(\frac{1}{1+k}\right)\mu_{a-aq} + \left(\frac{k}{1+k}\right)\mu_{a-mc} + \mu_{eof} \quad (1)$$

To follow peptide mobility as a function of micelle concentration and peptide charge (pH), it is helpful to correct for the influence of EOF on migration time. Therefore, we have defined the analyte effective mobility, μ_{eff}, as the apparent mobility minus the EOF mobility coefficient according to Eq. (2):

$$\mu_{eff} = \mu_{app} - \mu_{eof} \quad (2)$$

Arbitrarily, we define positive EOF to be flow from the injection end to the detection end of the capillary regardless of BGE or surfactant. As a result of the large EOFs generated in fused-silica tubes at pH>2.5, the observed or apparent mobility of all analytes is always positive. However, the electrophoretic mobilities of both the analyte (μ_{a-aq}) and analyte–micelle complexes (μ_{a-mc}) are negative because they are directed toward the injection end of the capillary. The effective mobility is, therefore, negative with respect to the EOF. To display a more intuitive picture of the effects of micelle concentration and pH on peptide migration, the absolute value of effective mobility, $|\mu_{eff}|$, is plotted. Consequently, an increase in absolute effective mobility reflects an increase in peptide–micelle interaction. The plots of absolute effective mobility of the pentapeptides as a function of SDS concentration at pH 8.8 and as a function of pH at 50 m*M* SDS are shown in Figs. 2 and 3, respectively. For example, peptide 2 formed the strongest complex with SDS

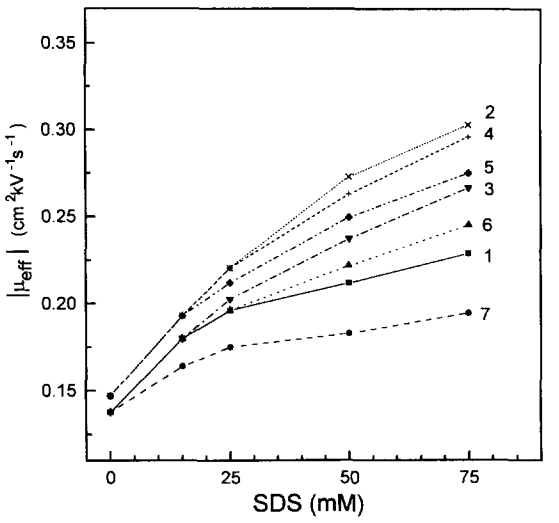

Fig. 2. Effect of SDS concentration on effective mobility (absolute value) of enkephalins and analogs in 50 m*M* sodium phosphate, pH 8.8, +11 kV.

micelles and thus had the largest mobility toward the injection electrode (Fig. 2).

At SDS concentrations above 25 m*M*, five of the peptides (Nos. 2, 4, 5–7) migrated in the order predicted by their hydrophobicity coefficients presented in Table 1. A continuous increase in effective mobility with SDS concentration was consistent with an increase in the ratio of micellar to aqueous phase. Peptide 7 was assumed to be the least hydrophobic analyte because of the sulfoxide moiety on the C-terminal methionine residue. Indeed, peptide 7 demonstrated the least interaction with the micelles and eluted closest to acetone, the EOF marker (Fig. 1). The five peptides (2, 4, 5–7) have a similar q/M_r ratio at pH 8.8; therefore, their migration behavior can be correlated to their extent of micelle complexation or interaction. Previous reports describing the difficulty in separating peptide analogs by SDS–MEKC [17,19] probably reflect the presence of non-specific electrostatic interactions of the analytes with the micelles. Fig. 3 showed similar non-specific yet strong interaction of the peptides with SDS micelles at pH 7.

Peptide 3 eluted earlier than predicted by its hydrophobicity coefficient (Table 1). Several explanations exist for the observed higher apparent mobility of peptide 3. First, peptide 3 has a smaller absolute q/M_r, hence lower free-solution mobility (μ_{a-aq}), compared with the other peptides, leading to a slight reduction in $|\mu_{eff}|$. Secondly, peptide 3 contains two D-alanine residues (D-ala^{2,3}), which might alter the backbone stereochemistry enough to hinder complex formation with the micelle. Evidence of this effect can be extrapolated from the results shown in Fig. 4, where the diastereomeric peptides 5 and 5′ were separated in pH 9.0 borate–SDS buffer with a difference in apparent mobilities of $\Delta\mu =$

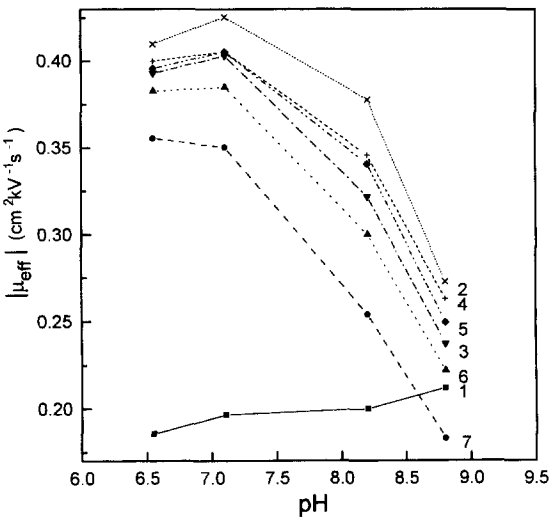

Fig. 3. Effect of background electrolyte pH on effective mobility (absolute value) of enkephalins and analogs in 50 m*M* SDS, 50 m*M* sodium phosphate, +11 kV.

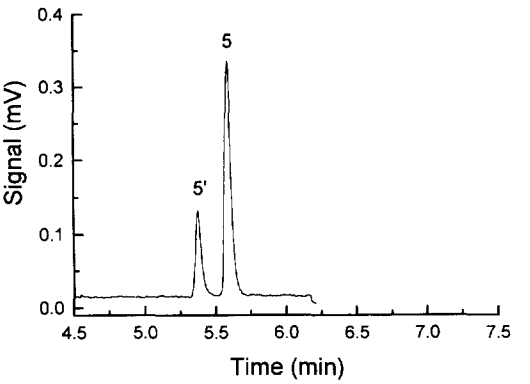

Fig. 4. MEKC electropherogram of peptide stereoisomers (see Table 1 for structures) separated in 50 m*M* SDS, 30 m*M* sodium tetraborate, pH 9 at +14 kV.

0.015 cm^2 kV^{-1} s^{-1}. The hydrophobicity of these peptides was predicted to be the same, yet peptide 5′, with the D-ala^2 residue, interacted less with the micelle than peptide 5. The difference in mobilities between peptides 5 and 5′ was almost twice the difference between peptides 5 and 3 (Fig. 2), within the experimental error (see Section 2.2). It seems that the D-ala substitution in ME at the second and third residues (i.e., peptide 3) may induce a conformation change, stabilized at pH 9, that diminishes peptide interaction with SDS micelles. For example, shielding of hydrophobic residues by the peptide backbone will presumably reduce the peptide–micelle hydrophobic interactions.

Hicks and co-workers showed, by molecular modelling and NMR studies of ME in 50 m*M* SDS at pH 4.1, results that are characteristic of a β-turn conformation that is stabilized by an intramolecular hydrophobic interaction of tyr^1 and phe^4, with little detectable insertion of the aromatic side chains into the hydrophobic core of the SDS micelles [22,26]. This conformation placed the N-terminal amine and all five carbonyl oxygens on an outer hydrophilic face and placed the amide protons on the inner hydrophobic face near the aromatic side chains. Their results suggest that electrostatic and hydrophilic interactions are primarily responsible for the retention of the enkephalin peptides, with a strong dependence on the conformation dictated by residues in the second and third position from the N-terminus. Our results for peptides 2–7 (Fig. 2) suggest that the hydrophobicity of the second and third residues induces a secondary or minor mechanism of interaction with SDS micelles. In other words, the migration order within the leu-terminal peptides (2,4) and the met-terminal peptides (3, 5–7) follows the relative hydrophobicity predicted in Table 1.

A peculiar behavior was seen for peptide 1, which was expected to be the most strongly complexed with SDS because of its D-trp^2 residue. If hydrophobic interaction with SDS micelles were truly secondary to electrostatic interaction, then peptide charge may play a more important role in explaining the migration behaviour of peptide 1. Fig. 3 shows the effect of pH on the absolute effective mobility for each peptide in phosphate–SDS buffer. The predicted acid dissociation constants for N-terminal tyr^1 are $pK_{a2}=7.36$ and $pK_{a3}=10.36$ [27]. Therefore, at pH 6.5, the fraction of each peptide having a positively charged N-terminus should be about 90%, with the overall net peptide charge being slightly negative. The large values of $|\mu_{eff}|$ at pH 6.5–7.2 for all analytes except peptide 1 (Fig. 3) must result from electrostatic and hydrophilic-based interaction of the peptide N-termini and the peptide carbonyl oxygens with the surface of the SDS micelles. Because electrostatic interactions are less specific than hydrophobic interactions for the peptides under study, the separation was observably worse at pH$<$ 7.5 than at pH$>$8.5. Consistent with the notion that the hydrophobic D-trp^2 residue may shield the neighboring amine group and therefore reduce pK_{a2} [25], peptide 1 carries less N-terminal positive charge than the other analytes over the range pH 6.5 to 8.5, resulting in less electrostatic interaction with the micelles. Analogous to shielding the N-terminal amino group, the D-trp^2 residue should cause a concomitant decrease in pK_{a3} of the tyrosine hydroxyl group to make peptide 1 a dianion at a pH lower than that for the other peptides. This onset of dianionic character is consistent with the observed slight increase in absolute effective mobility with increasing pH for peptide 1 (Fig. 3). However, the overall ionic effects do not fully explain the weak interaction of peptide 1 with the SDS micelles compared to the other seven peptides; therefore, additional steric effects must hinder complexation. It is important to note that the tryptophan side chain is highly conjugated (therefore, polarisable) and may be responsible for a weak hydrophilic interaction with the SDS micelles while at the same time masking the carbonyl oxygens from interaction.

3.2. CTAB–MEKC

Figs. 5 and 6 show the plots of absolute effective mobility of the seven peptides as a function of CTAB concentration at pH 4.1 and as a function of pH at 30 m*M* CTAB, respectively. Addition of ample cationic surfactant to the BGE is known to reverse the EOF in CE [17,31,32] because a bilayer is formed at the capillary wall. As a result of the reversed EOF and fixed-placement of the detector, the injection voltage polarity had to be changed from positive to negative. Nevertheless, we have defined positive EOF to be directed toward the detector,

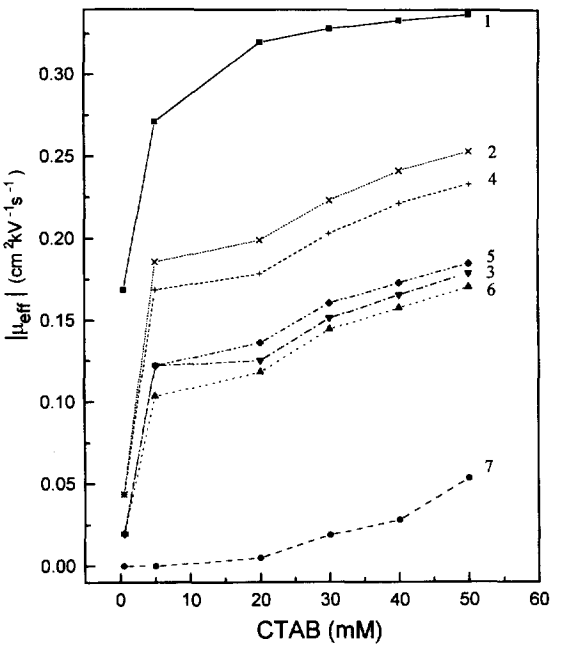

Fig. 5. Effect of CTAB concentration on effective mobility (absolute value) of enkephalins and analogs in 30 m*M* sodium phosphate, pH 4.1, −12 kV.

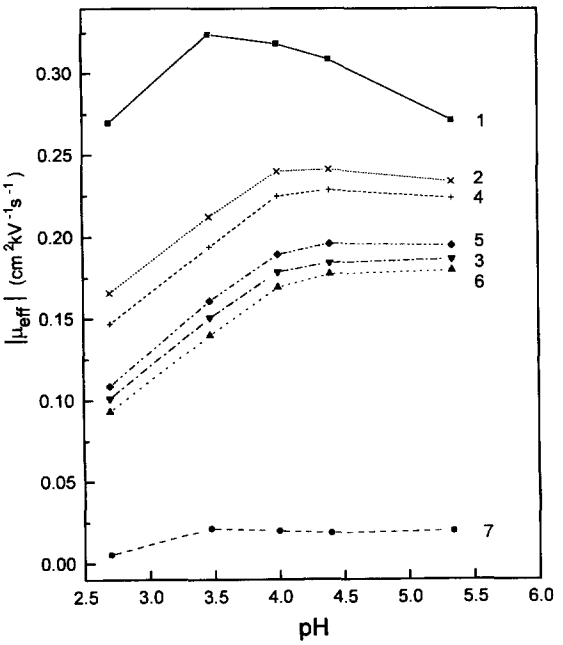

Fig. 6. Effect of background electrolyte pH on effective mobility (absolute value) of enkephalins and analogs in 30 m*M* CTAB, 30 m*M* sodium phosphate, −12 kV.

which is now at the anode end. At 0.5 m*M* CTAB, free-zone CZE conditions persisted because there was only enough CTAB to form the bilayer. Three closely eluting, broad peaks were seen at 0.5 m*M* CTAB (Fig. 5), which corresponded to the following groups of analytes: the leu^5 peptides (2, 4), the low-molecular-mass (M_r) met^5 peptides (3, 5, 6) and ME(O) migrating with the EOF marker. A sharper peak was recorded for D-trp^2-ME (peptide 1), which migrated the slowest and was therefore either the most positively charged or it experienced adsorption at the CTAB-modified capillary wall. These non-MEKC results suggest that peptide 7 is essentially neutral with a much lower isoelectric point (p*I*~4), under these experimental conditions, than originally expected. The hydrophobic shielding argument from above could not readily be applied to the C-terminal pK_a because peptides 1–6 were more positively charged than peptide 7, implying that they have a higher p*I* than peptide 7.

As the concentration of CTAB was raised, absolute effective mobility increased as expected (Fig. 5) and the migration order followed that predicted by the HPLC-derived hydrophobicity coefficients (Table 1), with the exception of peptide 3. Furthermore, the migration order of the pentapeptides in CTAB solution matched that for SDS, with the exception of peptide 1. The separation mechanism implied by Fig. 5 involves hydrophobic and electrostatic interaction because the peptides C-terminal residues still have a high proportion of negative character at pH 4.1. Although peptide 1 eluted in the order predicted by its hydrophobicity, it interacted the least with the CTAB micelles compared to the other analytes as shown by the smaller change in effective mobility, within experimental error, between 20 and 50 m*M* CTAB (Fig. 5). The high mobility of peptide 1 was presumably due to its high positive charge. The extent of micelle complexation was noticeably higher for CTAB compared to SDS for peptides 2–7. For example, doubling the surfactant concentration from 25 to 50 m*M* increased the absolute effective mobility by 20% with SDS versus 23% with CTAB for peptide 4 (LE), and by 13% with SDS versus 29% with CTAB for peptide 6 (ME). Conversely, peptide 1 interacted less with CTAB micelles compared to SDS micelles; the absolute effective mobility increased 8% when SDS concentration doubled but

increased only 4% when CTAB concentration was doubled. The influence of ionic strength on effective mobility was measured by increasing BGE at constant CTAB concentration and was found to be small compared to micelle interactions (results not shown). The relative viscosity with increasing surfactant concentration was not measured, but was assumed to contribute to the apparent mobility approximately equally for SDS and CTAB.

The plot of effective mobility versus buffer pH at 30 m*M* CTAB (Fig. 6) illustrates that the strength of electrostatic interaction increased as buffer pH was increased from 2.7 to 4. The absolute effective mobility of peptides 2–7 increased as expected; interactions with the cationic micelle increased with deprotonation of the C-termini. The much smaller increase in mobility for peptide 7 reflects its low propensity for interaction with the CTAB micelles. Peptide 1 showed an opposite trend to the other six peptides at pH>3.5 (Fig. 6). As buffer pH increased and peptide 1 became more negatively charged, we expected an overall increase in absolute effective mobility due to electrostatic complexation with the CTAB micellar phase. However, a decrease in absolute effective mobility with pH was seen. This mobility decrease is consistent with: (a) a decrease in free-solution electrophoretic mobility ($|\mu_{a-aq}|$) of peptide 1; (b) the small interaction with CTAB micelles observed in Fig. 5; and (c) the small interaction with SDS seen in Fig. 2. The CTAB results generally agreed with those from the SDS experiments in that the D-trp[2] residue in peptide 1 tended to inhibit interaction with the micelle compared to the other enkephalin analogs, contrary to what was predicted by the HPLC hydrophobicity coefficients. We assume that the D-trp[2] residue induces a particular peptide conformation that has neither a hydrophobic nor hydrophilic surface large enough to show a strong interaction with charged micelles.

4. Conclusions

The seven enkephalin analogs employed in this study showed appreciable peptide–micelle interaction with SDS and CTAB in aqueous solutions. However, the zwitterionic nature of the peptides over a wide pH range made it difficult to probe the hydrophobicity independently of electrostatic interactions. In general, the strength of the peptide–micelle interactions increased more with pH, in the direction of increasing opposite charge to the micelle, than with micelle concentration. This observation implies that hydrophobic interactions were fairly weak in comparison to the electrostatic and hydrophilic forces. Surfactants are readily used to solubilize proteins based on hydrophobic interactions; however, it is apparent that this mechanism is weak for the enkephalin analogs. In fact, our results suggest a hydrophilic interaction as the main source of peptide retention with SDS and CTAB micelles. Additionally, peptide conformation is a factor that must be considered when assessing the utility of MEKC as a probe for hydrophobicity. Molecular recognition of peptides by a receptor depends on selective interactions manifested in small changes in charge or hydrophobicity. To exploit separation techniques like CE and MEKC for modelling such biochemical processes, it will be necessary to better understand the molecular interactions that contribute to the selectivity in analyte migration. To this end, further studies are underway with other micellar systems and non-ionic micelles to better understand the role of the tryptophan residue on migration in MEKC.

Acknowledgments

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