Journal of Chromatographic Science, 2019, Vol. 57, No. 1, 54–62
doi: 10.1093/chromsci/bmy080
Advance Access Publication Date: 28 August 2018
Article

Article

Comparison of Chiral Recognition of Binaphthyl Derivatives with L-Undecyl-Leucine Surfactants in the Presence of Arginine and Sodium Counterions

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Received 6 January 2017; Revised 10 July 2018; Editorial Decision 30 July 2018

Abstract

In this study the chiral selectivity of L-undecyl-leucine (und-leu) for binapthyl derivatives was examined with the use of arginine and sodium counterions at pH's ranging from 7 to 11. The objective of this project was to investigate whether a cationic amino acid, such as arginine would achieve enhanced chiral selectivity when utilized as the counterion in the place of sodium in micellar electrokinetic chromatography. The data indicate that und-leu has significantly improved chiral selectivity toward 1,1′-binaphthyl-2,2′-diyl hydrogenphosphate (BNP) enantiomers in the presence of arginine counterions in comparison to sodium and that, at least in the case of this study, the enantiomeric form of the arginine did not appear to play a role in the chiral selectivity. The maximum resolution (Rs) achieved for BNP when sodium was used as the counterion was ~0.6. However, when arginine was used as the counterion, the maximum resolution for BNP was ~4.1. This was an increase in resolution of ~ 7-fold. However, no significant difference was observed for the enantiomers of 1,1′-bi-2-naphthol. In order to learn more about why this might be the case, NMR studies were conducted to examine what role the counterion might play in enantiomeric recognition.

Introduction

Among their many applications, amino acid-based surfactants are used in micellar electrokinetic chromatography (MEKC) to separate chiral analytes and study the mechanisms for chiral recognition (1–18). In a previous study, separation of 58 anionic, cationic and neutral enantiomers using undecyl-leucine-valine surfactant micelles was examined (1). In another study, separation of 12 chiral analytes was achieved using eight types of amino acid-based surfactants (3). A separate study examined the ability of N-undecylenyl-L-valinate to separate neutral, acidic and basic compounds (6). Other studies have investigated the effects of such factors as the number of amino acid moieties, amino acid order and the number of chiral centers in each surfactant on chiral selectivity (8–13). Some studies have also examined the effect of steric factors, polydispersity levels, solution temperature and the depth of penetration of analytes into the micellar core on enantiomeric recognition (13–18). In addition, NMR spectroscopy and molecular dynamics simulation studies have been utilized to examine the site of interaction of analyte enantiomers with amino acid-based surfactants (10, 19–23).

In a review of the literature, the authors were unable to identify any studies designed to examine the effect of counterion choice on enantioselectivity with amino acid-based surfactants utilized in

MEKC. Thus, the findings reported in this manuscript should help other separation scientist better understand the role counterions play in chiral recognition with amino acid-based systems. In addition, since enantiorecognition of the systems under study are amino acid-based, the knowledge gained from these studies will be potentially transferable to other amino acid-based systems such as proteins. For this study, the nature of chiral interactions in the amino acid-based surfactant L-undecyl-leucine (und-leu) was investigated through comparing the performance of arginine and sodium counterions. Arginine was chosen for this study because it has a cationic side chain, which is susceptible to predictable changes in ionization state when the solution pH is changed.

Experimental

Chemicals

L-leucine, L-arginine and D-arginine amino acids and racemic mixtures of 1,1'-bi-2-naphthol (BOH) and 1,1′-binaphthyl-2,2′-diyl hydrogenphosphate (BNP) were purchased from Sigma-Aldrich (St. Louis, MO). Undecyl L-leucine surfactants were synthesized from N-hydroxysuccinimide ester of undecylenic acid according to a previously reported procedure (8). The structures of these surfactants, analytes and arginine are provided in Figure 1.

Capillary electrophoresis procedure

The chiral separations were performed using a Hewlett–Packard (HP) 3D CE model #G7100A. The fused silica capillary [effective length of 45 cm (to detection window), 50-μm i.d., with a total length of 56 cm] was purchased from Agilent Technologies and mounted in a HP capillary cartridge. The temperature of the cartridge was maintained at 25°C throughout this experiment. Solutions of 50 mM und-leu with arginine and sodium were prepared in a 5 mM sodium borate buffer and the pH was adjusted to values of 7–11 with the use of NaOH and/or HCl. Note that unless otherwise stated, arginine refers to the L-enantiomer. These solutions were diluted to concentrations ranging from 15 to 50 mM and were filtered through a 0.45-μm filter before use. These concentrations (15 to 50 mM) of und-leu were examined because, as reported in a previous study, the CMC for und-leu in the presence of arginine is between 14 and 22 and for Na is between 17 and 21 for pHs 7.5–11.5 (24). A new capillary was conditioned for 30 min with 1 M NaOH, followed by 10 min with triple-distilled water. The capillary was then flushed with buffer containing the surfactant for 3 min prior to injection of the sample. Analyte standards were prepared in 1:1 methanol–water at 0.1 mg/mL. Samples were injected for 5 s at 10 mbar pressure. Separations were performed at + 30 kV, with UV detection at 230 nm.

NMR procedure

All NMR experiments were done at 25.0°C using a Bruker 400 MHz spectrometer. Diffusion coefficient, D, measurements were made with the bipolar pulse pair encode–decode pulse sequence (25). In these experiments, 24 NMR spectra were collected with magnetic field gradient pulses incremented from 0.8 to 20 G/cm. The gradient pulse duration, δ, was 4.0 ms, the diffusion time, Δ, was 250 ms, and the short delay, τ, between the bipolar gradients

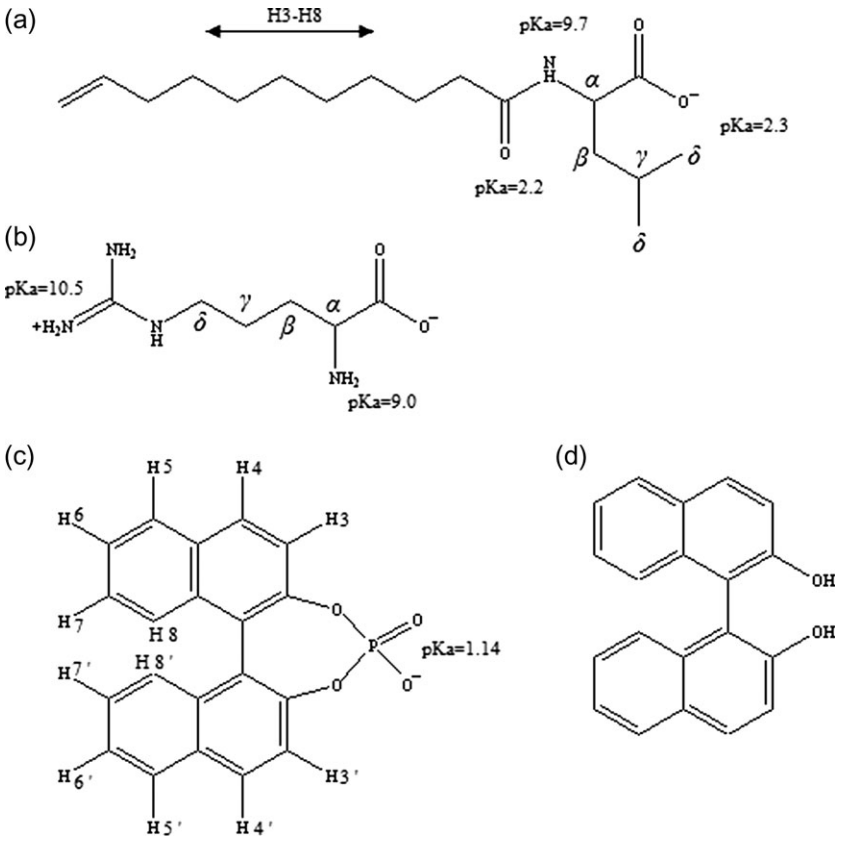

Figure 1. Structures of (a) und-leu, (b) arginine, (c) BNP and (d) BOH.

was 0.2 ms. The pulse sequence also used the WATERGATE method to suppress the solvent signal (26). Three replicate experiments were done with each sample and the spectral width of each spectrum was 6,173 Hz. After data acquisition, the intensities of the NMR resonances for each component in the mixture were recorded at each gradient value. Plots were then prepared of the natural log of peak intensity for each component vs the quantity $(\gamma \cdot G \cdot \delta)^2 \cdot (\Delta - \delta/3 - \tau/3)$. G is the magnetic field gradient strength and γ is the gyromagnetic ratio. A linear regression analysis was then performed and the slope of each plot was taken as $-D$.

Phase sensitive ROESY spectra were collected containing 2,048 and 256 data points in the F2 and F1 dimensions, respectively. Linear prediction analysis was then used to extend the data set in F1 by 200 points and zero filling was carried out to produce a 2048 × 1024 point data set. A $\pi/2$-shifted sine bell-squared apodization function was applied in both dimensions before Fourier transformation was carried out. A spin lock time of 200 ms was used in the ROESY experiments.

In the NMR diffusion experiments, the translational diffusion coefficient, D, was measured for each component in mixtures containing und-leu micelles with sodium and arginine as the counterions. The solutions were also spiked with a small amount of tetramethylsilane (TMS). The TMS molecules solubilized inside the und-leu micelles, allowing for the determination of the micelle diffusion coefficient, $D_{micelle}$, through an analysis of the TMS peak's decay with increasing magnetic field gradient strength (27). This value was then substituted into the Stokes–Einstein equation to calculate the hydrodynamic radius, R_h, of the und-leu micelles (27). Since the und-leu surfactant molecules undergo fast exchange between the micelle and free solution, the diffusion coefficient reported by the und-leu resonances equation 1, $D_{obs,und\text{-}leu}$, is the weighted average of the micelle bound, D_{mic}, and free solution, $D_{und\text{-}leu,free}$, values (24).

$$D_{obs,und-leu} = f_{b,und-leu} * D_{mic} + (1 - f_{b,und-leu}) D_{und-leu,free} \quad (1)$$

$D_{free,und\text{-}leu}$ is the und-leu diffusion coefficient in a free solution, which was found to be $(6.04 \pm 0.02)\text{x}10^{-10}$ m^2/s and $f_{b,und\text{-}leu}$ is the mole fraction of und-leu molecules bound to micelles (24).

In an analogous fashion, arginine and sodium ions bound to the und-leu micelles also undergo fast exchange between bonded and free solution states. Therefore, the diffusion coefficient calculated for the counterion resonances, $D_{obs,\ arg}$ is given by equation 2 (27). $D_{free,arg}$ is the free solution diffusion coefficient for arginine, $(8.10 \pm 0.02)\text{x}10^{-10}$ m^2/s and $f_{b,arg}$ is the mole fraction of arginine molecules bound to the micelles (24).

$$D_{obs,arg} = f_{b,arg} * D_{mic} + (1 - f_{b,arg}) * D_{free,arg} \quad (2)$$

Similarly, BNP enantiomers experience fast exchange between free solution and micelle-bound states as well. Therefore, the observed diffusion coefficient for BNP, $D_{obs,BNP}$, is given by equation 3 (25–28).

$$D_{obs,BNP} = f_{b,BNP} * D_{mic} + (1 - f_{b,BNP}) * D_{free,BNP} \quad (3)$$

$D_{free,BNP}$ is the free solution BNP diffusion coefficient, $(6.63 \pm 0.01)\text{x}10^{-10}$m^2/s, and $f_{b,BNP}$ is the mole fraction of BNP molecules bound to the micelles. Finally, BNP–micelle association constants, K, were calculated with equation 4, where [und-leu] is the und-leu surfactant concentration in mol/L (24).

$$K = \frac{f_{b,BNP}}{(1 - f_{b,BNP})[\text{und} - \text{Leu}]} \quad (4)$$

In this study, diffusion experiments were performed with solutions containing und-leu micelles, and either Na^+ or arginine counterions. Measurements were made at pH 8.0.

Results

This study investigates the effect of solution pH on the chiral recognition of und-leu in the presence of sodium and arginine as counterions. As observed in Figure 2, at pH ~7 the average hydrodynamic radius was ~14 Å when arginine is the counterion but decreases to ~10 Å at pH 11. This same phenomenon was not observed when sodium was the counterion. As can be seen in the same figure (Figure 2), the hydrodynamic radius remains relatively constant when sodium was the counterion (~9.5 Å) from pH 7.5 to ~10. After pH 10, a slight increase in hydrodynamic radius was observed but that change is relatively small compared with the radius when arginine was the counterion. The purpose of this study was to compare sodium and arginine counterions and amino acid-based surfactant's chiral interaction with binapthyl derivatives. The enantiomers of BNP and BOH were separated in MEKC using und-leu and either sodium or arginine as counterions at various pH's and surfactant concentrations. The pH range for this study was between 7 and 11 and the concentration of und-leu was 15–50 mM. Below pH 7, und-leu is not soluble therefore we did not report any solutions below pH 7 and we also did not report any solutions above pH 11 because of the strong EOF and the high current limited the MEKC separations. Resolution of BNP and BOH enantiomers are shown in Figures 3 and 4, respectively. Figure 3 clearly indicates that at low pH, in the presence of the arginine as the counterion, und-leu provides better chiral recognition for enantiomers of BNP. However, und-leu surfactant provides slightly better chiral separation for enantiomers of BOH in the presence of sodium counterion (see Figure 4). Shown in Figure 5 is a comparison of the retention factors (k') as a function of pH, surfactant concentration and counterion for BNP and BOH. As can be seen in Figure 5, the retention factors for BOH and BNP follow very similar trends. At lower pH values, the retention factors of BOH and BNP were higher with arginine as the counterion compared to when sodium was the counterion. As

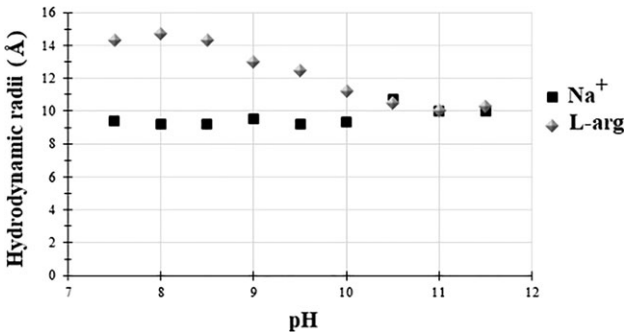

Figure 2. Hydrodynamic radii (Å) of und-leu micelles as a function of pH and counterion. Average std = ±0.1.

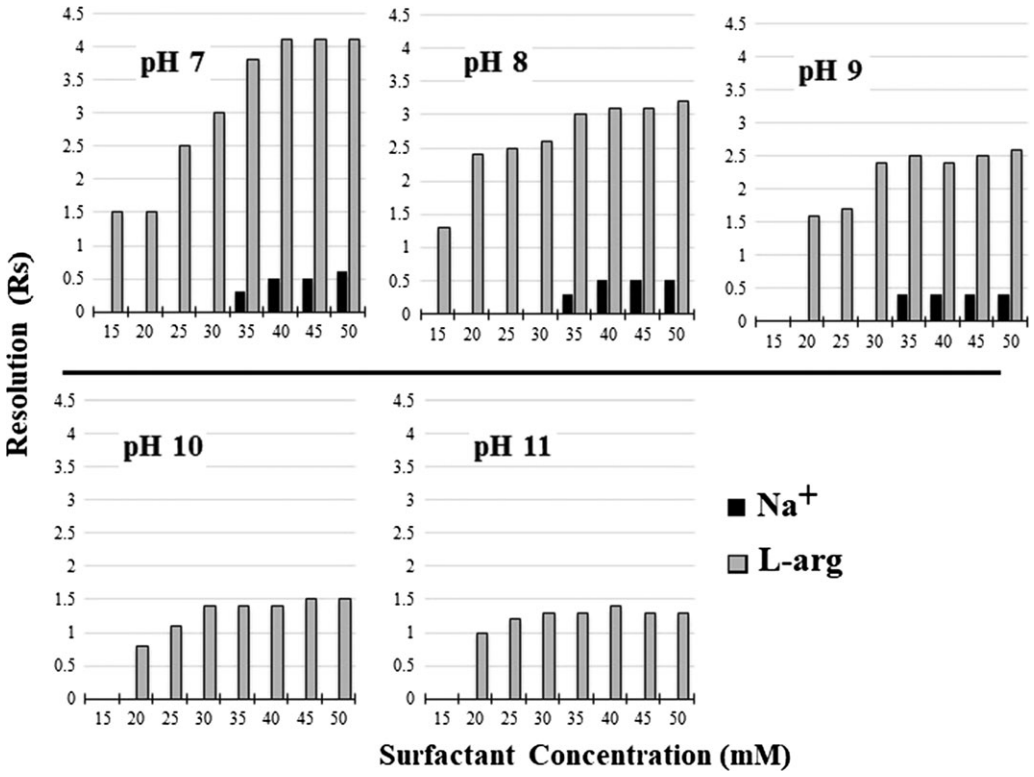

Figure 3. The resolution of (a) BNP enantiomers (b) BOH with the use of Na+ and L-arg counterions, at concentrations of und-leu of 15, 20, 25, 30, 35, 40, 45 and 50 mM and at pH's of 7–11. Detection is at 230 nm, applied voltage was at +30 kV. Average std= ± 0.1.

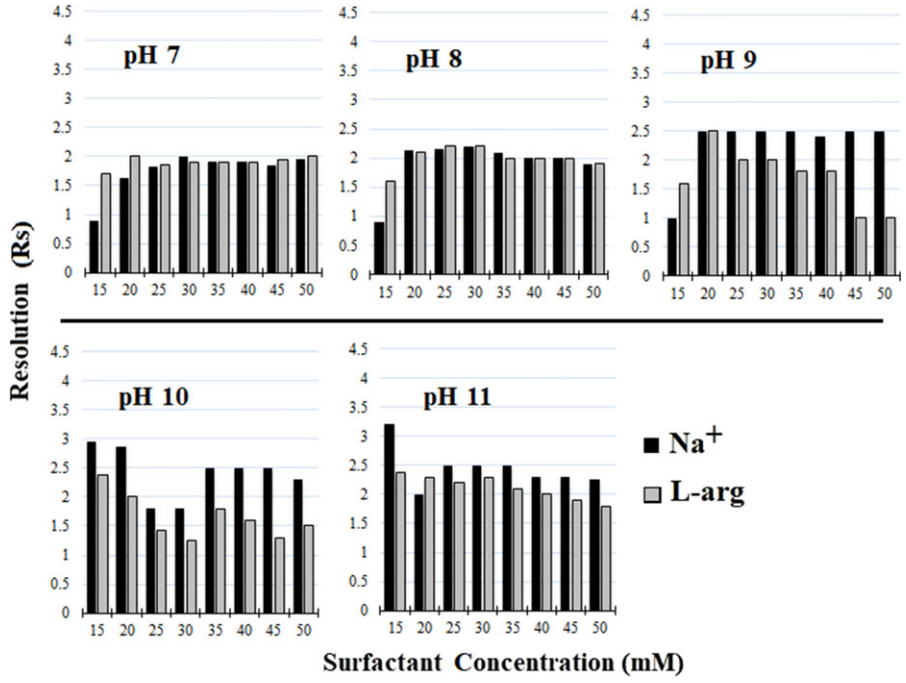

Figure 4. The resolution of BOH enantiomers with the use of Na+ and L-arg counterions, at concentrations of und-leu of 15, 20, 25, 30, 35, 40, 45 and 50 mM and at pH's of 7–11. Detection is at 230 nm, applied voltage was at +30 kV. Average std = ±0.1.

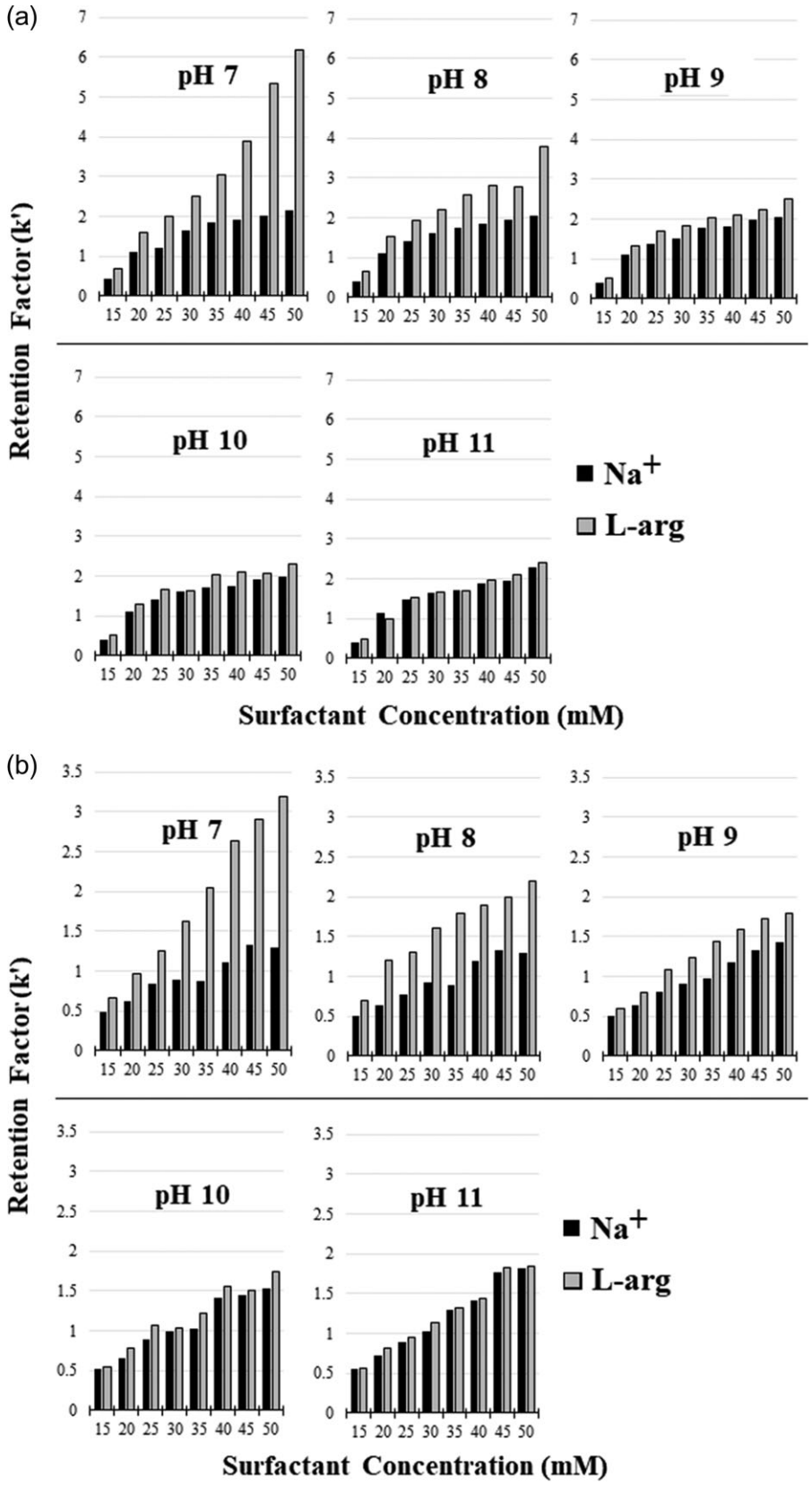

Figure 5. Comparison of *k'* (a) BOH (b) BNP with the use of Na+ and L-arg counterions, at concentrations of und-leu of 15, 20, 25, 30, 35, 40, 45 and 50 mM and at pH's of 7–11. Detection is at 230 nm, applied voltage was at +30 kV. Average std= ± 0.03.

the pH increases, the differences in the retention factors decrease. In fact, at pH 11 the retention factors are, for all practical purposes, the same. A similar trend was also observed when examining the effect of pH and type of counterion on the size of the und-leu micelle (Figure 2).

In order to try to gain further insight into the role arginine might play in the enhanced chiral recognition observed compared to when sodium was used as the counterion, various NMR experiments were conducted. NMR diffusion experiments were performed to collect information about diffusion coefficients, along with fraction bound (f_b) values for the analytes and BNP–micelle association constants for mixtures containing 50 mM und-leu and 50 mM $NaHCO_3$ at pH 8.0. These results are shown in Table I(a). Overall, the Table I (a) results show that the binding of (R)-BNP and (S)-BNP to und-leu micelles containing Na^+ cations is very similar. Diffusion coefficients, f_b values, hydrodynamic radius and micelle association constants are reported in Table I(b) for solutions containing und-leu micelles, arginine counterions and BNP enantiomers at pH 8.0. In these experiments, the micelle radius and the fraction of und-leu and arginine molecules bound to the micelles are very similar in the solutions containing (R)-BNP and (S)-BNP. The Table I(b) results, however, show that the BNP fraction bound values for the two enantiomers are slightly different: 0.950 and 0.958 for (R) and (S)-BNP, respectively. This result translates into different micelle association constants for (S)-BNP (460) and (R)-BNP (380). Given the precision of the NMR diffusion measurements and the small difference between the diffusion coefficients measured for (R)-BNP and (S)-BNP, these K values are only reported to two significant figures. Table I(b) also shows that the magnitude of the $f_{b,BNP}$ values and micelle association constants are larger in solutions containing arginine counterions than in solutions containing Na^+.

In order to gain further insight into BNP association with und-leu micelles in the presence of arginine counterions, a Rotating Frame Overhauser Enhancement Spectroscopy, or ROESY, spectrum was collected for an und-leu/(S)-BNP/arginine mixture. ROESY is a two-dimensional NMR technique in which cross-peaks connect NMR resonances for protons that are within ~5 Å of one another. Intramolecular ROESY cross-peaks connect protons within the same molecule, while intermolecular cross-peaks connect protons that are close to one another, but belong to different molecules. Figure 1 shows structures of und-leu, arginine and BNP with atom labels used in the ROESY analyses.

Discussion

The chiral recognition of amino acid-based surfactants is largely based upon electrostatic, hydrophobic, steric interactions and hydrogen bonding. These forces in return effect the depth at which an analyte penetrates into the micellar core. Previous studies have shown that neutral hydrophobic analytes tend to bind at a greater depth into the micellar core while hydrophilic analytes interact closer to the surface of the micelle. Enantiomers of BOH that are mostly neutral at pH's below 9 interact closer to the core of amino acid-based micelles while negatively charged enantiomers of BNP interact closer to the surface (12). Also, surfactants with amino acid headgroups have both amide and carboxylic acid functional groups and therefore, solution pH would be expected to affect the percent ionization of the carboxylic groups and the rate at which amide protons exchange with the solvent (24). The degree of headgroup ionization and amide proton solvent exchange rate would in return be expected to affect depth of the penetration of the analyte into the micellar core and thus chiral recognition.

This study investigates how pH can affect chiral separation of binapthyl derivative enantiomers in the presence of und-leu surfactant and arginine counterion. The structure of arginine and the pK_a of its side chain are shown in Figure 1. Most arginine molecules are positively charged at pH's below its side chain pK_a, which makes this amino acid a good candidate for the counterion of negatively charged und-leu surfactant. Also, arginine is capable of forming hydrogen bonds with a chiral analyte as well as the leucine functional groups. In addition, solution pH can affect arginine counterions more than sodium. As can be seen in Figure 2, the pH affects the size of the und-leu micelles in the presence of arginine. As mentioned before, pH does not significantly affect the size of the und-leu in the presence of Na. These findings suggest that at higher pH's arginine more easily dissociates from the micelles due to the loss of positive charge that occurs after this change in pH environment.

These findings are supported by a previous study which reported that the percent of arginine counterions bound to the und-Leu micelle ($f_{b,arg} \cdot 100$) drops from ~39% at pH 7–9 to ~10% at pH 11 (24). In that study, it was also shown that arginine Hδ protons and und-Leu Hδ methyl groups were close to one another, confirming that arginine cations bind to the surface of und-Leu micelles rather than the interior. In addition, an NMR ROESY experiment showed that there were interactions between the und-leu headgroup protons

Table I. Diffusion Coefficients, Hydrodynamic Radii, Fraction Bound Values and Enantiomer Association Constants for Solutions Containing Und-Leu Micelles and Na^+ Counterions and Und-Leu Micelles and L-Arg Counterions at pH 8.0

(a) und-leu and Na^+ counterions

$D_{und\text{-}leu} \times 10^{10}$ (m^2/s)	$D_{BNP} \times 10^{10}$ (m^2/s)	$D_{micelle} \times 10^{10}$ (m^2/s)	R_h (Å)	f_b, und-leu	f_b, BNP	K_{BNP}
(R)-BNP						
2.44 ± 0.03	2.50 ± 0.01	2.11 ± 0.01	10.2 ± 0.1	0.916 ± 0.010	0.913 ± 0.004	210
(S)-BNP						
2.47 ± 0.01	2.53 ± 0.01	2.14 ± 0.01	10.0 ± 0.1	0.915 ± 0.008	0.912 ± 0.004	210

(b) und-leu and L-arg counterions

$D_{und\text{-}leu} \times 10^{10}$ (m^2/s)	$D_{BNP} \times 10^{10}$ (m^2/s)	$D_{L\text{-}arg} \times 10^{10}$ (m^2/s)	$D_{micelle} \times 10^{10}$ (m^2/s)	Rh (Å)	f_b, und-leu	f_b, L-arg	f_b, BNP	K_{BNP}
(R)-BNP								
1.87 ± 0.01	1.63 ± 0.01	4.52 ± 0.01	1.37 ± 0.01	15.7 ± 0.1	0.892 ± 0.006	0.532 ± 0.003	0.950 ± 0.002	380
(S)-BNP								
1.77 ± 0.01	1.60 ± 0.01	4.54 ± 0.02	1.38 ± 0.01	15.6 ± 0.1	0.916 ± 0.006	0.529 ± 0.004	0.958 ± 0.003	460

and both the arginine Hα and Hγ protons and that the amino acid cation interacted with und-leu micelles through both of its amine functional groups (Figure 6). Since arginine interacts with the polar head of und-leu surfactants, chiral recognition may be affected by such interactions. In this study, the effect of pH on the chiral recognition of BOH and BNP enantiomers was investigated with und-leu in the presence of arginine and sodium as counterions.

Separation of BNP enantiomers

The enantiomers of BNP were separated in MEKC using und-leu and either sodium or arginine as counterions at various pH's and surfactant concentrations. As can be seen in Figure 3, when arginine was used as the counterion for the separation of BNP, the enantiomeric resolution increased as a function of surfactant concentration from ~15 mM to around 30–40 mM where the separations, reported as resolutions, began to level off. Clearly, arginine is a significantly better counterion for the enantiomeric separation of BNP compared to sodium. When sodium was used as the counterion, the enantiomeric resolution of BNP never exceeded 0.6. However, when arginine was the counterion resolutions as high as 4.1 were achieved. In addition, as can be seen from Figure 3, the worst enantiomeric resolution (Rs = 0.8 at pH 10 for 20 mM und-leu) were achieved when arginine was used as the counterion is better than the highest enantiomeric resolution (Rs = 0.6 at pH 7 for 50 mM und-Leu) achieved when sodium was the counterion. In Figure 7, electropherograms of BNP separation are compared at pH 7 with sodium (Figure 7A) and arginine (Figure 7B) as counterion. While no separation of BNP enantiomers are observed at 20 mM und-Leu surfactants in the presence of Na as a counterion, baseline separation of BNP enantiomers are achieved at same pH and und-Leu concentration in the presence of arginine as a counterion.

In regard to the trend displayed between buffer pH and the chiral recognition of BNP in the presence of arginine, resolution is likely to decline with an increase in pH due to a resultant loss of positive charge in the arginine side chains which causes the dissociation of arginine from the micelles (see Figure 1 for pKa). Previous studies on the fraction bound (f_b) of Arginine to und-leu support the likelihood of this interaction, as measures of fraction bound (f_b) decrease with increasing pH. At the pH range of 7–9, f_b remains near 39%, while this measure decreases to ~28% at pH 9.5 and ~10% at pH 11.5 (24).

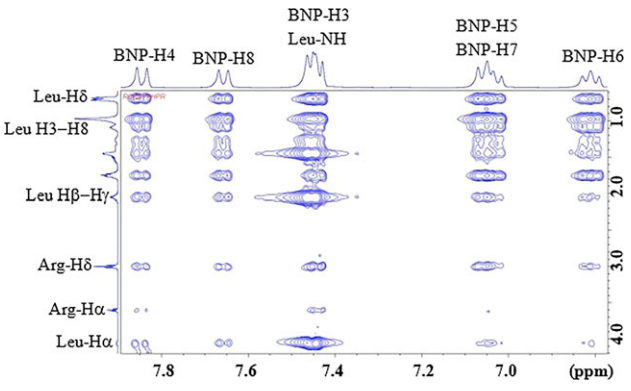

Figure 6. ROESY spectrum showing intermolecular cross-peaks between (S)-BNP and und-leu and L-arg protons.

Separation of BOH enantiomers

For BOH, chiral separation may be said to be roughly equal in magnitude when either sodium or arginine counterions were used with sodium possibly being a slightly better counterion (see Figure 4). In looking at the effect of und-leu concentration on the enantiomeric separation of BOH, no predictable associations are shown to occur, though there are some notable patterns. For und-leu-Na, the maximum resolution (Rs = 3.2) occurred at pH 11 and at a 15 mM concentration of the buffer while for und-leu-arg, the maximum resolution (Rs = 2.5) occurred at pH 9 and at 25 mM. These results indicate that at pH's 7–9, optimum resolution is observed around 20 mM und-leu-Na. In looking at the chiral selection of BOH by und-leu-arg, a decline in resolution from 2.5 to 1.0 occurred at pH 9 from 20 to 50 mM. A similar decline was observed at pH 10 and 1.1. However, the difference for pH 11 was not significant. At pH 11, the resolution declined from 2.3 to 1.8 from 15 mM to 50 mM.

It is worth noting that in order to rule out the possibility that arginine was forming aggregates in solution leading to enhanced chiral recognition, a control was run with 50 mM arginine in 5 mM sodium borate. No chiral separation was observed with either BNP or BOH.

Comparison of retention factors (k′) for BOH and BNP

Shown in Figure 5 is a comparison of the retention factors (k') as a function of pH, surfactant concentration and counterion for BNP and BOH. As can be seen in Figure 5, the retention factors for BOH and BNP follow very similar trends. At lower pH values, the retention factors of BOH and BNP are higher with arginine as the counterion compared to when sodium is the counterion. As the pH increases, the differences in the retention factors decrease. In fact, at pH of 11 the retention factors are, for all practical purposes, the same. A similar trend was also observed when examining the effect of pH and type of counterion on the size of the micelles formed. As previously discussed and as shown in Figure 2, at low pH's the size of the micelles formed when arginine was the counterion are larger than when sodium was the counterion. In fact, the size of the micelle when sodium was the counterion is relatively constant with changes in pH, while the size of the micelle decreases with an increase in pH when arginine was the counterion.

Both of these phenomena (changes in k' and micelle size) as a function of pH and counterion are likely due to changes in electrostatic attraction between the micelle and the counterions as a function of pH. As discussed previously, arginine has two amines with the α-amine having a pK_a of ~9.8 and the pK_a on the side chain amine being ~10.5. Therefore at a pH of 9.8, the α-amine is around 50% neutral and at a pH of ~10.8 it has lost most (~90%) of its charge. The decrease in the positive charge on the α-amine causes a decrease in the overall electrostatic attraction between arginine and the negatively charged carbocyclic group on the und-leu surfactant headgroup.

Another very important issue worth pointing out is that since the retention factors for BNP at higher pH values are practically the same for both counterions, then the enhanced chiral selectivity obtained when arginine is the counterion is not due to an increase in k' but rather a change in the chiral interactions with BNP when arginine is the counterion. It is also worth pointing out that the enhanced chiral selectivity is likely due to a change in the chiral pockets being formed rather than chiral interactions between BNP and arginine. In order to determine if the enhanced chiral selectivity was due to chiral interactions of BNP with arginine, we conducted

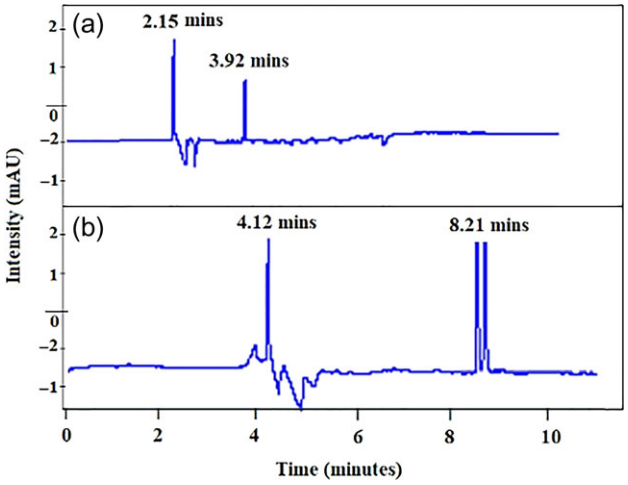

Figure 7. Chiral separation of BNP enantiomers with 20 mM und-leu, 5 mM sodium borate buffer at pH 7, detection at 230 nm, and 30 kV applied voltage and 25°C. (a) Na+ and (b) L-arg as a counterion.

experiments with both the L and D form of arginine with an enantiomeric excess of the R-enantiomer of BNP. In this experiment (data not shown) we did not observe a reversal of enantiomeric order, nor a decrease in the enantiomeric resolution. Thus, it can be concluded that the increase in chiral recognition is likely not due to the chirality of arginine.

NMR Studies

NMR ROESY was utilized to better understand the interaction of und-leu with BNP enantiomers. Figure 6 shows the expansion of the ROESY spectrum for a solution containing (S)-BNP, arginine and und-leu micelles at pH 8.0. The Figure 6 expansion shows (S)-BNP and the und-leu NH resonance across the F2 axis and und-leu and arginine resonances across the F1 axis. Many of the cross-peaks in Figure 6 spectrum connect BNP and und-leu resonances. For example, the und-leu Hα resonance at 4.06 ppm, the und-leu Hβ and Hγ resonances at 1.45 ppm, the und-leu hydrocarbon chain resonances at 0.97 ppm and the und-leu Hδ resonance at 0.62 ppm each connect to all six BNP peaks. Figure 6, however, also shows intermolecular cross-peaks between the arginine Hα proton at 3.00 ppm and weaker cross-peaks between the BNP resonances and the arginine Hα proton at 3.41 ppm. Und-leu peaks overlap the arginine Hβ and arginine Hγ resonances, so no unambiguous intermolecular arginine–BNP cross-peaks could be assigned to these arginine protons. However, the fact that intermolecular ROESY cross-peaks were observed between the BNP protons and arginine Hα and Hδ confirms that both BNP and arginine are bound to the micelle surface and are close enough (i.e., within ~5 Å) to give ROESY cross-peaks. Therefore, the ROESY results suggest that as mentioned above, both BNP enantiomers bind and arginine counterions bind near the surface of the und-leu micelles.

Conclusion

The results of this study suggest that when arginine is utilized as the counterion in the place of sodium with und-leu surfactants, a pronounced increase in chiral resolution is observed for BNP enantiomers. However, the same effect was not observed for BOH. In addition, in support of previous findings, the results of this study suggests pH can have a relatively significant effect on micelle size and shape. In this case, the type of counterion played a significant role in the changes in the micelle size and shape as a function of pH. As reported in this manuscript, at pH ~7 the average hydrodynamic radius for und-leu micelles was ~14 Å when arginine is the counterion but decreases to ~10 Å at pH 11. This same phenomenon was not observed when sodium was the counterion. When sodium was used as the counterion, the hydrodynamic radius remains relatively constant (~9.5 Å).

Finally, although arginine was demonstrated to be a better performing counterion for the separation of BNP, it is significant that the chirality of arginine was found to have little impact on chiral selectivity. Further studies will be conducted with various diamine compounds with varying hydrocarbon chain length spacers to learn more about this effect

Acknowledgments

We also acknowledge the generosity of the Ralph E. Klingenmeyer family. Z. R. summer stipend was provided by McNair Scholarship.

Funding

This work was supported by an National Science Foundation- Research in Undergraduate Institutions grant (#1213532) to Dr. Fereshteh Billiot and Dr. Kevin Morris, a National Science Foundation-Major Research Instrumentation grant (#1531526) to Dr. Fereshteh and Eugene Billiot, a Robert A. Welch Chemistry Departmental Grant to the Chemistry Program at Texas A&M University-Corpus Christi, a HUMAA (Howard University Medicine Alumni Association) Endowed Founder's Chair in Basic Science award to Dr. Yayin Fang and a National Institute of Heath-National Institute of Minority and Heath Disparities grant (#G12 MD007597) to the Research Centers in Minority Institutions Program at Howard University.

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