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Depth of penetration of binaphthyl derivatives into the micellar core of sodium undecenoyl leucyl-leucinate surfactants

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Abstract

Two different diastereomeric forms of sodium *N*-undecanoyl leucyl-leucinate (SULL) (both L,L and L,D) are used to examine the role of depth of penetration of chiral analytes into the micellar core of polymeric and monomeric surfactants on enantioselectivity. In this study, chiral separation of three binaphthyl derivatives, i.e. (±)-1,1′-bi-naphthyl-2,2′-diamine (BNA), (±)-1,1′-bi-2-naphthol (BOH), and (±)-1,1′-binaphthyl-2,2′-dihydrogen phosphate (BNP), are studied. Chromatographic results suggest that BNP interacts approximately the same with both the C- and N-terminal amino acid of poly SULL, while the preferential site of interaction of this analyte with the monomeric form of SULL (mono SULL) is at the C-terminal amino acid. This indicates that BNP enantiomers penetrate deeper into the micellar core of the poly SULL than that of the mono SULL. Varying the temperature resulted in a change in the depth of penetration of BNP into the micellar core of the poly SULL. However, the enantiomers of BNA and BOH always interact preferentially with the N-terminal amino acid of SULL surfactants (both polymer and monomer), independent of the temperatures studied. © 2002 Published by Elsevier Science B.V.

Keywords: Enantiomer separation; Hydrophobicity; Diastereomers; Depth of penetration; Surfactants; Dipeptide; Binaphthyl derivatives

1. Introduction

Chiral separations using chiral polymeric pseudo-stationary phases (CPSP) were first reported in the literature by the Warner [1] and Dobashi [2] groups around 1994. Covalent stabilization in polymeric surfactants results in a more rigid structure that may lead to unique chiral selectivity as compared to the monomer equivalents. Since the report of the first chiral separations with polymeric CPSP, several manuscripts and review articles have been published regarding the use and understanding of the mechanism of the chiral separations with these polymers [3–11].

Billiot et al. have proposed that the depth of penetration of the analytes into the micellar core of the polymeric dipeptide surfactants plays a significant role in chiral recognition [3]. The authors concluded that some of the factors that dictate the depth of penetration of the chiral analyte into the micellar core are hydrophobicity, steric factors, and

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the effective charge on the analyte, as well as the surfactant. In that study, the authors also stated that hydrophobic neutral analytes, in general, interact preferentially with the N-terminal amino acid of the polymeric dipeptide surfactants under study. Due to electrostatic interactions, cationic enantiomers interact preferentially with the C-terminal amino acids of anionic dipeptide surfactants. However, moderately hydrophobic analytes interact with both (C- and N-terminal) amino acids [3].

It should also be noted that the hydrophobicity of the analyte, as well as the hydrophobicity of the running buffer, plays a major role in analyte–selector interaction [12]. The hydrophobicity of the running buffer in electrokinetic chromatography (EKC) can easily be varied by either changing the separation temperature or adding organic modifiers. In addition to its influence on hydrophobicity, temperature can also significantly affect the kinetic, thermodynamic, and electromigration processes in EKC separations [13].

In EKC, chiral separation is achieved due to differences in interaction of the two enantiomers with the CPSP. These differences are due to the formation of transient diastereomeric complexes between the CPSP and chiral analytes. In order for chiral separations to be achieved, the energy of formation of the diastereomeric complexes must be different for the two enantiomers. Temperature plays a significant role in the formation and stability of these complexes [5]. For example, temperature may shift the pK_a of the CPSP, as well as the enantiomers. This in turn alters the electrostatic interactions. Temperature may also change the structure of the selector and/or the analyte. Change in spatial shape of the complexes may produce variations in the electrophoretic mobilities and chiral interactions. For example, proteins which have been used extensively as CPSP undergo structural changes at different temperatures [14]. Furthermore, from an electrokinetic view, viscosity and electroosmotic flow are temperature dependent. In this manuscript, we focus on another aspect not commonly examined with respect to temperature; depth of penetration of the analyte into the hydrophobic core of the polymeric CPSP.

In a recent report by our group, the chromatographic performances of polymeric and monomeric surfactants were studied [15]. That study concluded that the rigidity of the polymeric surfactants may limit penetration of some analytes into the micellar core of the polymeric surfactants, as compared to that of the monomers. However, some analytes may penetrate deeper into the core of the polymers as compared to monomers. In this manuscript, we investigate the role of temperature on the depth of penetration of binaphthyl derivatives into the micellar core of monomeric and polymeric surfactants.

2. Experimental

2.1. Chemicals

Leucine–leucine, (±)-1,1′-bi-naphthyl-2,2′-diamine (BNA), (±)-1,1′-bi-2-naphthol (BOH), and (±)-1,1′-binaphthyl-2,2′-dihydrogen phosphate (BNP) were purchased from Sigma (St. Louis, MO, USA). Sodium borate, tris (hydroxymethyl) aminomethane (TRIS), and methanol were obtained from Fischer Scientific Company (Fair Lawn, NJ, USA).

2.2. Synthesis of polymeric dipeptide chiral surfactants

Undecenoyl leucyl-leucinate surfactants (SULL) were synthesized from the *N*-hydroxysuccinimide ester of undecylenic acid according to a previously reported procedure [16]. The acid forms of these surfactants were then converted to the sodium salt by addition of equimolar concentrations of sodium bicarbonate in the presence of methanol. The sodium salt of SULL was then obtained by evaporating the solvent and freeze-drying. A 100-m*M* solution of SULL was polymerized by use of γ-radiation (^{60}Co; 70 Krad/h) for about 7 days (total dose; 3–4 Mrad). Proton NMR spectroscopy was used to follow the polymerization process. At the conclusion of polymerization, the NMR spectra did not show the proton signals for the vinyl protons.

2.3. Capillary electrophoresis procedure

Electrokinetic chromatography (EKC) separations were performed by use of a Hewlett-Packard (HP) 3D CE model #G1600AX. The fused-silica capil-

lary, effective length of 55 cm (to detection window), 50 μm I.D., with a total length of 63.5 cm, was purchased from Polymicro Technologies (Phoenix, AZ, USA) and mounted in an HP capillary cartridge. Each new capillary was conditioned for 30 min with 1 N NaOH at 60 °C, followed by 10 min with triply distilled water. The capillary was then flushed with buffer for 2 min prior to sample injection.

A buffer containing 10 m*M* sodium borate and 100 m*M* TRIS was prepared in triply distilled water; 30 equimolar monomeric concentration of surfactants were added and the pH was adjusted to 10 by addition of either HCl or NaOH. All analyte standard solutions 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 220 nm. The EKC conditions for optimum enantiomeric resolution have been previously determined [6,15].

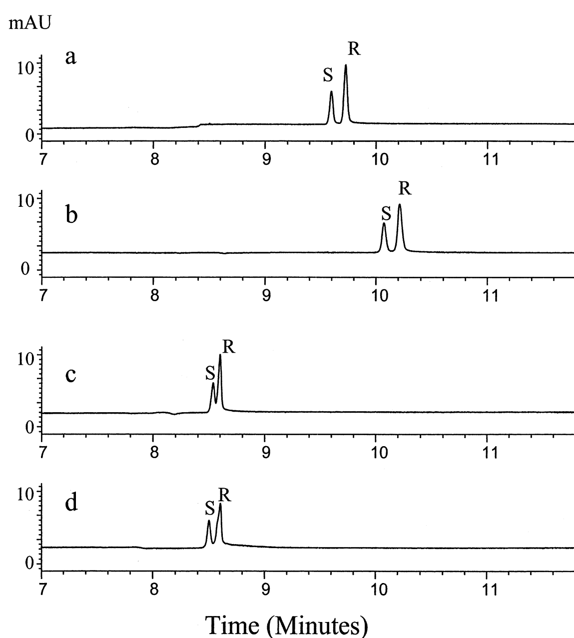

Fig. 1. Enantiomeric separation of BNA. (a) poly L,L-SULL; (b) mono L,L-SULL; (c) poly L,D-SULL; (d) mono L,D-SULL.

3. Results and discussion

In this study, differences in chiral selectivity of the monomeric and polymeric surfactants were observed. Based on previous studies, we have concluded that these differences are probably due to variations in hydrophobicity and rigidity of the surfactants [3]. Hydrophobic forces largely dictate the depth of penetration of a chiral analyte into the micellar core. In order to compare the depth of penetration of chiral analytes into the micellar core of polymeric and monomeric surfactants, enantiomers of BNA, BOH, and BNP were separated using diastereomeric surfactants (in L,L and L,D configuration) of SULL. In L,L-SULL surfactants, both amino acids have L-configurations, while in L,D-SULL, the N-terminal amino acid has an L-configuration and the C-terminal amino acid has a D-configuration.

3.1. Enantiomeric separation of binaphthyl derivatives

Fig. 1 shows the chiral separation of BNA using various SULL surfactants. Both, poly and mono L,L-SULL separated the enantiomers of this analyte with a resolution value of around 1.8 (Fig. 1a–b). However, as shown in Fig. 1c–d, mono L,D-SULL provided a resolution value of 1.4 while poly L,D-SULL resolved the enantiomers of BNA with a resolution value of only 1.0.

As noted from the electropherograms shown in Fig. 2a–b, both poly and mono L,L-SULL resolved the enantiomers of BOH equally well. As with BNA, mono L,D-SULL provided better resolution for BOH enantiomers than poly L,D-SULL. This may be due to the greater ability of the monomer to relax its configuration and allow deeper penetration of the enantiomers of these analytes (BNA and BOH) into the micellar core of the monomer as compared to the polymeric form of SULL. These results suggest that BNA and BOH enantiomers interact more strongly with the C-terminal amino acid of poly L,D-SULL as compared to mono L,D-SULL. Since the C-terminal amino acid of L,D-SULL has the opposite configuration of the N-terminal amino acid, it is reasonable to assume that the more an analyte interacts with both chiral centers on the dipeptide surfactant with different chiral centers (i.e. L,D-SULL) the lower the chiral resolution. In addition, it should be noted that in the electropherograms shown in Figs. 1 and 2, the *S*-

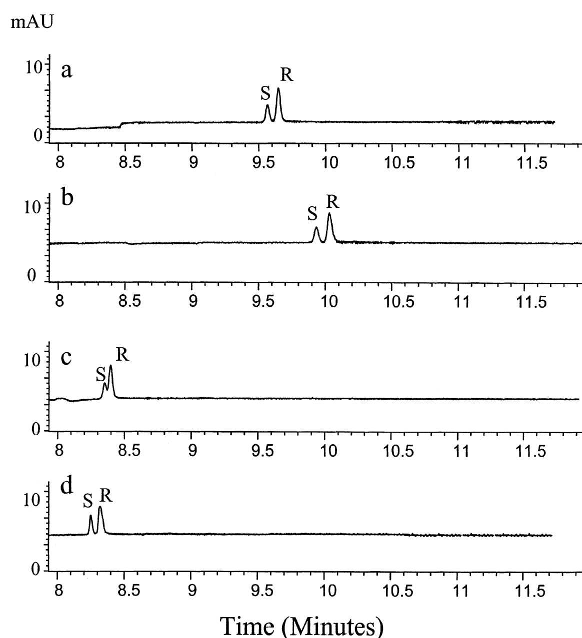

Fig. 2. Enantiomeric separation of BOH (a) poly L,L-SULL; (b) mono L,L-SULL; (c) poly L,D-SULL; (d) mono L,D-SULL.

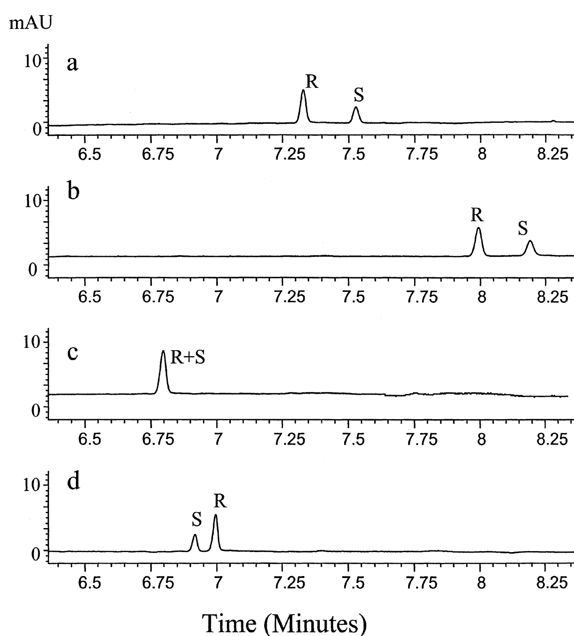

Fig. 3. Enantiomeric separation of BNP (a) poly L,L-SULL; (b) mono L,L-SULL; (c) poly L,D-SULL; (d) mono L,D-SULL.

enantiomer, which is at half the concentration of the *R*-enantiomer, always eluted first. From this migration order, it is reasonable to assume that the *R*-enantiomer interacts more strongly with both polymeric and monomeric L,L-SULL, than does the *S*-enantiomer.

The enantiomeric separation of BNP with monomeric and polymeric SULL is illustrated in Fig. 3. Poly L,L-SULL provided a separation of the enantiomers of BNP with a resolution value of 5.8 (Fig. 3a) which is slightly better than mono L,L-SULL (Rs 5.5), Fig. 3b. According to the chromatographic data displayed in Fig. 3, the *S*-enantiomer of BNP interacts more strongly with both polymeric and monomeric L,L-SULL, than the *R*-enantiomer. Separation of BNP with L,D-SULL is shown in Fig. 3c–d. As illustrated in Fig. 3c, polymeric L,D-SULL does not separate the enantiomers of BNP. This is probably due to similar BNP interaction with both chiral centers of poly L,D-SULL (which are of opposite chiral conformation). Interestingly, monomeric L,D-SULL separates the enantiomers of this analyte with a resolution value of 2.3.

Under the conditions used for this study (pH 10), BNP enantiomers are negatively charged; therefore, this analyte is more soluble in the buffer than either BNA or BOH. However, the presence of four fused benzene rings on BNP provided rigidity and hydrophobicity as well. These competing factors, hydrophobicity of the aromatic groups, and hydrophilicity of the anionic phosphate group dictates the site of chiral interaction of BNP with the surfactant. Since no enantiomeric separation of BNP was observed with poly L,D-SULL and a reversal of enantiomeric order was observed with mono-L,D-SULL as compared to mono- and poly-L,L-SULL, it is reasonable to assume that the preferential site of interaction of BNP with mono-L,D-SULL is closer to the bulk aqueous phase as compared to poly-L,D-SULL. In other words, BNP interacts preferentially with the C-terminal amino acid of monomeric surfactants and approximately the same with the C- and N-terminal amino acids of poly SULL.

Our above hypothesis is consistent with the migration order of the enantiomers of BNP observed with the monomer and polymer of this surfactant. The reversal of enantiomeric order of BNP with mono-L,D-SULL as compared to mono- and poly-L,L-SULL

indicates that the *R*-enantiomer of BNP interacts preferentially with the C-terminal amino acid (D-configuration) of mono-L,D-SULL. In contrast to BNP, enantiomers of BOH and BNA are highly hydrophobic. Therefore, these enantiomers penetrate more deeply into the core of the monomeric CPSP as compared to BNP. Note that the migration order of *R*- and *S*-BOH and BNA with the polymers and the monomers of L,L-SULL (Figs. 1a,b and 2a,b) is the same as for L,D-SULL (Figs. 1c,d and 2c,d). This suggests that the enantiomers of BOH and BNA probably interact preferentially with the N-terminal amino acid of the dipeptide CPSP (both polymeric and monomeric form). Examination of Fig. 4a–b suggests the respective proposed preferential site of interaction of these analytes with polymeric and monomeric SULL. Although BNA and BOH enantiomers interact preferentially with the N-terminal amino acids, they also interact to some extent with the C-terminal amino acid. This is evident from an increase in chiral recognition of these enantiomers with the corresponding polymers and monomers of L,L-SULL as compared to L,D-SULL.

3.2. Effect of temperature on chiral separation of binaphthyl derivatives

The enantioseparation of BNP at three different temperatures is shown in Fig. 5, using poly L,D-SULL as the CPSP. At 12 °C, slight separations of *R*- and *S*-BNP was achieved. At intermediate temperature, i.e. 25 °C, no chiral recognition of the BNP was observed, while at higher temperature, i.e. 55 °C, again, some chiral selectivity was obtained. Note, in the electropherogram shown in Fig. 5a, *S*-BNP, which is at half the concentration of *R*-BNP, elutes first, whereas at 55 °C (Fig. 5c) the *S*-enantiomer elutes second. In other words, varying the temperature resulted in reversal of the migration order of the BNP enantiomers. At low temperature, the BNP enantiomers interact preferentially with the N-terminal amino acid of poly L,D-SULL. This is probably

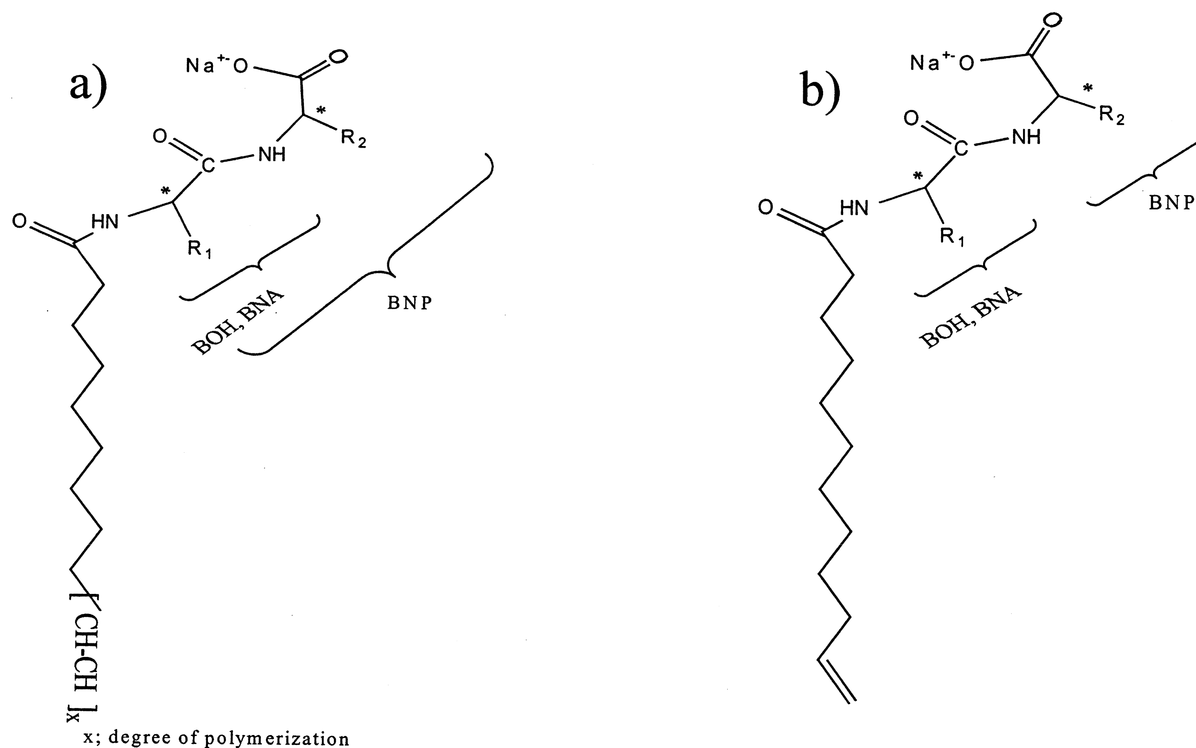

Fig. 4. Site of interaction of BNA, BOH and BNP with: (a) poly SULL and (b) mono SULL.

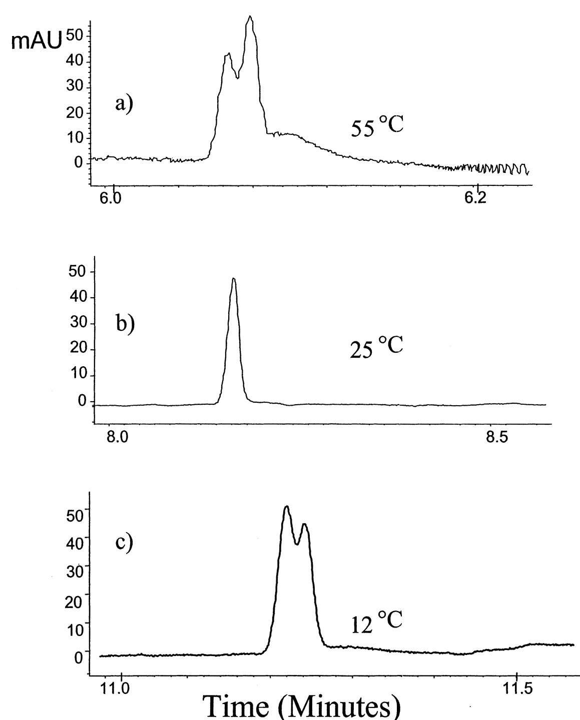

Fig. 5. Enantioseparation of BNP in different temperatures.

because BNP is less soluble in the bulk solution at lower temperatures; therefore, it penetrates more deeply into the micellar core of the poly-SULL surfactants. By increasing the temperature, this analyte becomes more soluble in the bulk solution. At intermediate temperature, BNP interacts similarly with both amino acids of poly L,D-SULL. Finally, at 55 °C, the BNP enantiomers interact preferentially with the C-terminal amino acid. Investigation of BOH and BNA enantiomers at different temperatures indicates that the enantiomers of these analytes preferentially interact with the N-terminal amino acid of the polymeric SULL surfactants at all temperatures examined.

4. Conclusion

The depth of penetration of the analyte into the micellar core of dipeptide surfactants determines which chiral center(s) the analyte preferentially interacts. Among the analytes investigated in this study, BOH and BNA preferentially interact with the N-terminal amino acid of the SULL surfactants (both monomeric and polymeric), while the enantiomers of BNP interact preferentially with the C-terminal chiral center of the monomeric and both chiral centers of the polymeric SULL. Varying the temperature of the running electrolyte resulted in a change in the depth of penetration of the BNP enantiomers. At low temperature, BNP interacts with the N-terminal chiral center, while at higher temperatures, it interacts with the C-terminal chiral center of poly-SULL. In contrast, BNP interacts with both chiral centers of poly-SULL surfactant at intermediate temperature.

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