Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1080 (2005) 140–147
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Enantiomer separation by reversed-phase liquid chromatography with novel hydrophobic phases composed of chiral cationic surfactants

Kaori Kurata *, Junji Ono, Akira Dobashi

Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachiouji, Tokyo 192-0392, Japan

Received 15 November 2004; accepted 29 April 2005
Available online 23 May 2005

Abstract

This paper describes enantiomer separation using four kinds of chiral stationary phases (CSPs) where quaternary ammonium surfactants containing L-valine diamide moieties into long alkyl chains were bound to silicagel supports by reversed phase liquid chromatography. Our aim was to examine hydrogen bonding association of the chiral moiety in hydrophobic phase brought about by aggregation of the micelle-forming surfactants on the surface. The following CSPs were thus derived from the vinyl-terminated chiral surfactants via hydrosilylation: CSP **1** from *N*-[3-(10-undecenoyl-L-valylamino)propyl]-*N*,*N*,*N*-trimethylammonium bromide, CSP **2** from *N*-[6-(10-undecenoyl-L-valylamino)hexyl]-*N*,*N*,*N*-trimethyl-ammonium bromide, CSP **3** from *N*-[3-(10-undecenoyl-L-valylamino)propyl]-*N*-octadecanyl-*N*,*N*-dimethyl-ammonium bromide and CSP **4** from *N*-[6-(10-undecenoyl-L-valylamino)hexyl]-*N*-octadecanyl-*N*,*N*-dimethylammonium bromide. The degree of hydrophobicity in the interfacial phase was observed by measuring pyrene fluorescence in aqueous media including an organic modifier. Retention of racemic *N*-acylleucine isopropyl esters was highest in CSP **4**, followed by **3**, **2**, and **1**. Largest α values toward enantiomer separation were observed for CSP **4** where the chiral moieties were kept through a hexamethylene unit apart from the polar head groups and to which another long alkyl chain was attached, as compared with those for CSP **4**. In CSP **4**, the chiral moiety to interact with enantiomeric solutes should be buried into the interfacial phase deeply in more extent than CSP **3**. In a similar manner, CSP **2** has more effective for enantiomer separation than CSP **1**. The interfacial phase of these CSPs was easily exposed to the bulk phase because of the affinity between the bulk phase and the polar head groups as well as their electrostatic repulsion. However, degree of the enantiomer separation can be controlled by the depth of the chiral moiety in the hydrophobic interfacial phase.

Keywords: Enantiomer separation using aqueous media; Reversed phase liquid chromatography; Cationic surfactant; Half lipid structure

1. Introduction

Liquid chromatography (LC) using chiral stationary phases (CSPs) is one of the most sophisticated means for detecting chiral recognition ability of transient diastereomeric intermolecular association. This can be recognized as enantiomer separation when the chiral molecule is bound to solid support and the enantiomer is sorbed to the support from the mobile phase [1,2]. Hydrogen bonding is one of the most significant contributors to form diastereomeric associations between the enantiomers [3]. Through the preliminary study, it was revealed that hydrogen-bond associations are weakened in reversed phase liquid chromatography with aqueous media because of their strong polarities to prevent interactions between CSP and the enantiomeric solutes. Using acetyl-L-valine *tert*-butylamide as a model compound for the chiral selection with silicagel modified with (10-undecenoyl)-L-valine *tert*-butylamide via hydrosilylation (CSP **5**), the hydrogen bonding associations between this region and 4-nitrobenzoyl(NB)-L-leucine isopropyl ester were measured using nuclear magnetic resonance (NMR) techniques [3]. Hydrogen bonds at the two amide sites of the chiral diamide moiety confirmed enantioselectivity in both the normal and reversed phases. CSP **5** showed enantioseparability and proved the effectiveness of hydrogen-bond association in aqueous media [4]. The chiral separation of enantiomers should thus occur when such hydrogen bond-

* Corresponding author. Fax: +81 426 70 7067.
E-mail address: kurata@ps.toyaku.ac.jp (K. Kurata).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.04.095

ings are formed in a hydrophobic environment, shielded from the bulk aqueous phase. This was also demonstrated by the fact that chiral micelles formed by *N*-[3-(dodecanoyl-L-valylamino)propyl]-*N*,*N*,*N*-trimetylammonium bromide (surfactant **1a**) and *N*-[6-(nonanoyl-L-valylamino)hexyl]-*N*,*N*,*N*-trimetylammonium bromide (surfactant **2a**) were capable of separating enantiomers in aqueous media by electrokinetic chromatography (EKC) [5,6]. In EKC, these micelles, formed in dynamic association-dissociation equilibrium with monomeric surfactants in water [7,8], functioned as a transient stationary phase entrapping enantiomers by hydrophobic interactions. Micellar EKC (MEKC) was known as method of chiral purity determination in micro-scale.

Immobilization of micellar structures on solid supports is thus thought to be an effective method to separate substrates using a combination of hydrophobic interaction and hydrogenassociation in LC with aqueous media. Aqueous LC using phosphatidylcholine-bonded silicagel has been found to selectively separate small hydrophobic peptides both with and without cysteine residues [9,10]. This chromatographic technique, in which half of the lipid bilayer is immobilized on the solid support, is based on the hydrophobic effects of the hydrocarbon chain in phosphatidylcholine and the additional electrostatic and steric interactions of its polar head group. In this study, *N*-[3-[(10-undecenoyl)-L-valylamino]propyl]-*N*,*N*,*N*-trimethylammoniumbromide (surfactant **1b**) and *N*-[6-(10-undecenoyl-L-valylamino)hexyl]-*N*,*N*,*N*-trimethylammonium bromide (surfactant **2b**), which are congeners of **1a** and **2a**, respectively, were chemically bound to silicagel in order to produce a hydrophobic interfacial phase composed of the local aggregation of these surfactants on the silicagel surface. These stationary phases were CSP **1** and **2**, respectively. The surfactants grafted onto the surface are expected to stretch due to the associations between the polar head group and the bulk water. On the other hand, at least in part, the bonded surfactant molecules should be folded and/or collapsed on the surface, as has been cited as random walk of surfactants into micelles; thus leading to make up hydrophobic interfacial phases based on their aggregation under aqueous media. Under these conditions, the difference in the depth with which the valine diamide moiety is located in the interfacial phases of CSP **1** and **2** should be observed in the enantiomer separation with aqueous mobile phase solvents.

N-[3-(10-Undecenoyl-L-valylamino)propyl]-*N*-octadecanyl-*N*,*N*-dimethylammonium bromide (surfactant **3a**) and *N*-[6-(10-undecenoyl-L-valylamino)hexyl]-*N*-octadecanyl-*N*,*N*-dimethylammonium bromide (surfactant **4a**), in which a long hydrocarbon chain was added to surfactants **1b** and **2b**, respectively, were considered to provide vesicle structures and be more hydrophobic around the valine diamide moieties compared to surfactants **1b** and **2b**, from which they were derived. These surfactants were thus chemically bound to silicagel to obtain CSP **3** and **4**, which have interface structures similar to half of the lipid bilayer structure.

The hydrophobic environment brought about by aggregation of surfactants bonded on silicagel surface was evaluated with pyrene whether it has enough hydrophobicity and volume to form hydrogen-bond association between valine diamide moieties and solutes in aqueous media. The chiral selectivity of CSPs **1**–**4** was evaluated using benzoyl, 3,5-dinitrobenzoyl (DNB), and 4-nitrobenzoyl (NB) amino isopropyl esters as model enantiomers, separated using mobile phases composed of water–organic solvent mixtures.

2. Experimental

2.1. Preparation of chiral cationic surfactants and surfactant-bonded silicagels

Three chiral cationic surfactants capable of forming micelles, surfactants **1a**, **1b** and surfactant **2a**, and CSP **5** were prepared as previously reported [6].

*2.2. CSP **1** from N-[3-(10-undecenoyl-L-valylamino) propyl]-N,N,N-trimethylammonium bromide (surfactant **1b**)*

CSP **1** was prepared by binding surfactant **1b** to silicagel by hydrosilylation, according to the procedure previously reported [6].

*2.2.1. N-[3-[(11-chlorodimethylsilylundecanoyl) valylamino]propyl]-N,N,N-trimethyl-ammonium bromide (**1c**)*

Surfactant **1b** (778 mg) and a catalytic amount of chloroplatinic acid (5 mg) were dissolved in 5 mL of dry chloroform. To the solution was added 3 mL dimethylchlorosilane, after which the mixture was refluxed 60 °C under an argon atmosphere. After 20 h, a 0.5 mL portion of the mixture was extracted, evaporated to dryness using a vaccum and the residue was redissolved in 0.5 mL of deuteriochloroform. The completion of the hydrosilylation was confirmed using ^{1}H NMR to monitor the disappearance of the vinyl protons signal in surfactant **1b**. The solvent was then removed under reduced pressure from the original solution, the residue was redissolved in dry chloroform, and the solvent was subsequently evaporated. This procedure was repeated twice in order to completely remove the excess silane reagent. The residue was used in the following step without further purification.

*2.2.2. CSP **1***

Silicagel [1.25 g; Nucleosil 100-5, 5 μm (specific surface area, 350 m^2/g), Macherey-Nagel, Dueren, Germany] was dried at 180 °C for 20 h under reduced pressure and then cooled to room temperature. The silicagel was suspended in 3 mL of freshly distilled anhydrous pyridine. The silylated surfactant **1c** was dissolved in 5 mL of anhydrous pyridine, and subsequently added to the suspension. The mixture was gently stirred at room temperature for 20 h. The