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Molecular recognition with micellar and micelle-like aggregates in aqueous media

Akira Dobashi*, Masaki Hamada

Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji Tokyo 192-03, Japan

Abstract

In this article, attention is directed to molecular recognition by micellar aggregates made with ionic surfactants involving directed interactions of substrates. Particular emphasis is placed on chiral recognition of enantiomers by hydrogen bonding functionalities incorporated in hydrophobic micellar interior. Hydrophobic properties within micelles, the ordering of their polar headgroups containing chiral functionalities essential for the recognition and the cessation of micellar kinetic association–dissociation with polymerization and immobilization of the surfactants on the support are discussed. © 1997 Elsevier Science B.V.

Keywords: Reviews; Molecular recognition; Enantiomer separation; Amino acids

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1. Introduction

Aqueous micellar aggregates provide a hydrophobic pseudophase distinct from bulk water, in which selective interactions of substrates may occur through their properly oriented functionalities [1–3]. Surfactants having polar headgroups and non-polar hydrocarbon tails are associated with hydrophobic interactions to form micelles that are in dynamic association–dissociation equilibrium with monomeric surfactants in bulk water [3,4]. Hydrophobic interactions are relatively non-directional and thus

*Corresponding author.

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the recognition of molecular shape and chirality requires intermolecular force directed toward the substrates. Hydrogen bonds contribute significantly to molecular recognition in non-polar solvents, as observed for various host molecules in the selective entrapment of neutral molecules by directed hydrogen bonds [5–13]. These bonds, however, are weak in water unless they act, at least to some degree, as a cooperative array as in the case of biological macromolecules. A simple means for making hydrogen bonds effective in water would thus be to incorporate the bonding elements into a micelle which would shield them from bulk water.

Molecular recognition with micellar aggregates possessing binding affinity toward substrates is discussed in the following. Through the use of micelles produced with chiral surfactants and micelles containing chiral auxiliaries, the chiral recognition of enantiomers has been shown possible. Chromatography using chiral stationary phases (CSPs) is an effective means for detecting energy differences in diastereomeric complexes formed through interactions of host molecules and enantiomers [14–16]. In electrokinetic chromatography (EKC), to assess micellar potential for molecular recognition [17–21], study was made of micelles constituting a pseudo-stationary phase to which substrates are distributed from aqueous mobile phase in a capillary tube. In this micellar phase, hydrophobic interactions contribute primarily to substrate retention.

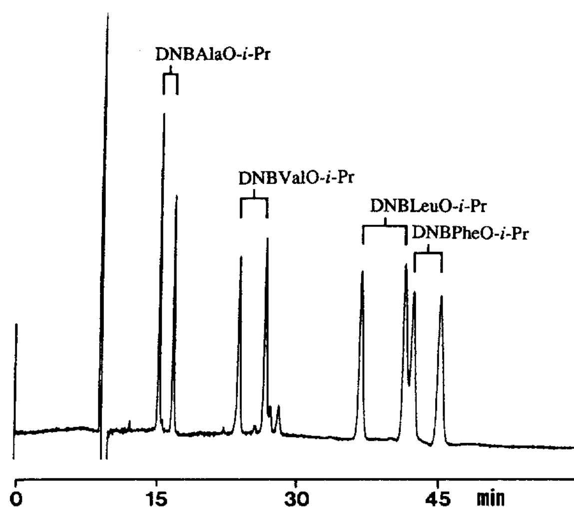
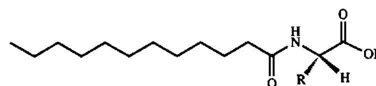


Fig. 1. Separation of a mixture of four enantiomeric 3,5-dinitrobenzoyl (DNB) amino acid isopropyl esters with 1a micellar solution by electrokinetic chromatography. Conditions: column, fused-silica tubing 50 cm×50 mm I.D.); micellar solution, 0.025 *M* 1a in 0.025 *M* borate–0.05 *M* phosphate buffer (pH 7.0); total applied voltage, ~12.4~12.8 kV; constant electric current, 40 μA; detection, UV at 254 nm; temperature, ambient.

1.1. Recognition by micelles with chiral anionic surfactants

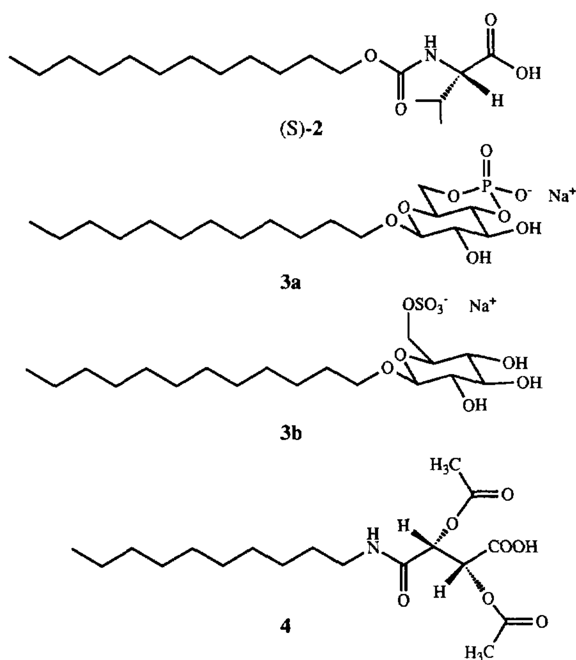
Micelles comprised of anionic N-dodecanoyl-L-valine (1a) sodium salts are capable of resolving enantiomers of amino acid derivatives with EKC [22–24]. The hydrogen bonding affinity of chiral amides incorporated into these surfactants has been shown effective for the chiral recognition of enantiomers inside the hydrophobic micellar interior. Fig. 1 shows the separation of a series of N-3,5-dinitrobenzoyl (DNB) amino acid isopropyl esters through the use of micelles formed by 1a alone in phosphate–borate buffer. This solute derivatization is the most effective for the enantiomer separation and the D-enantiomer was found to elute more rapidly than the corresponding L-enantiomer in all cases, indicating chiral micelles to bind more to the L-enantiomer with configuration the same as that of the chiral surfactant than to the D counterpart. Enantiomeric DNB-amino acids could not be resolved in this study with anionic micelles. The retention times were virtually the same as those under the same conditions except when 1a surfactant was not present in the buffer solution. These acids are thus negatively charged in buffer solution (pH 7.0) and electrophoretically migrate toward the positive end of the column without entering anionic micelles. The mechanisms of interactions between micelles and substrates shown in Fig. 2 should be clarified [25].



(S)-1a, R = - $CH(CH_3)_2$; 1b, R = - CH_2OH; 1c, R = - CH_2CH_2COOH; 1d, R = - CH_3

Surfactant 1a functions to provide a hydrophobic environment in which bulk water is excluded within the micellar interior and make hydrogen binding possible to substrates [24]. Many chiral surfactants

with these functions have been used in EKC; their skeleton types are not only those of N-acylamino acids derived from various amino acids [22–24,26–30] but also those of N-acyloxyvalines (2) [31–33], alkyl β-glucopyranosides (3) [34], N-acyldiacetyltartaric acid monoamides (4) and bile salts such as sodium deoxycholate and sodium taurodeoxycholate [35–38]. Among the chiral surfactants, bile salts exhibit aggregation behavior different from that of other long-chain alkyl surfactants owing to their hydrophobic and hydrophilic faces and are thus referred as micelle-like aggregates.



In the traditional model of aggregation of bile salts, there is the stepwise formation of initial primary micelles each consisting of 2–8 monomers held together by hydrophobic interactions between bile salts non-polar faces [3]. Micelles of bile salts may however possibly be represented by a helical model at least in the case of conjugated bile salts such as sodium taurodeoxycholate [39].

For the ionic alkyl surfactants, globular micelles with a chiral headgroups associated with the water surface and with a hydrocarbon tail separated from the water surface due to hydrophobic effect should be easily imaged. The aggregation number of 1a as

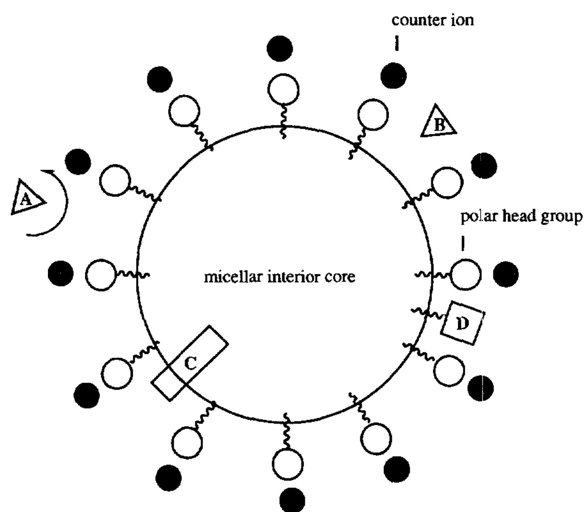
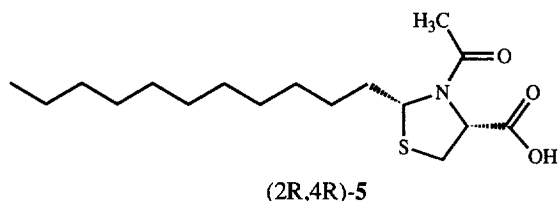


Fig. 2. Possible solubilization modes of an aqueous micelle. A charged solute (A) may possibly be electrostatically repelled from the micellar surface of the same charge-type as the ionic micelle, while an oppositely charged solute (B) may be electrostatically attracted to the micellar surface. Non-polar solutes (C) may partition to the more hydrophobic core region near the Stern layer. Amphiphilic solutes (D) may take on alignment to maximize their electrostatic and hydrophobic interactions with surfactants.

determined from light-scattering data in the phosphate–borate buffer was 498. 1a should thus form non-spherical micelles since this value far exceeds the maximum possible aggregation number expected from the hydrocarbon chain length of 1a on the bases of assumption of micellar sphericity [40]. This lack of sphericity may also hold true of above all alkyl surfactants containing branched bulky headgroups.

1a micelles are effective for the recognition of phenylthiohydantoin (PTH) derivatives of amino acids [26]. Addition of SDS, urea and methanol to 1a solutions were found in this study to improve peak shape and resolution between enantiomers in EKC. These additives have been used with micelles formed by sodium salts of N-dodecanoyl-L-serine (1b) [27] and N-dodecanoyl-L-glutamic acid (1c) [28,29]to resolve PTH derivatives. Among N-acyl-L-amino acid salts, 1b micelles were found to have interfacial structures ordered with hydrogen-bonding network formed between amide units of headgroups by circular dichroism (CD) spectrum study [41]. The surfactant derived from cysteine (5) has a characteristic structure containing a thiazolidine ring and

was effective for separating racemic drug mixtures of cromakalin and fenoldopam in EKC [30].



(2R,4R)-**5**

With N-dodecoxycarbonylvaline (2) considerably similar to 1a surfactant, the enantiomer separation of many pharmaceutical amines such as ephedrine and homatropine was carried out effectively [31]. The micelles of 2 gave better separation of enantiomers compared to 1a micelles. Extensive separation by 2 micelles has been demonstrated for N-benzoylamino acid methyl esters [32] and 6-aminoquinoyl-N-hydroxysuccinimidyl carbamate derivatives of amino acids [33]. In the case of 2, there is less background absorption in the shorter UV wavelength region, so that more sensitive detection should be possible. This was observed in the case of anionic surfactants containing D-glucose residues such as dodecyl β-D-glucopyranoside monophosphate (3a) and monosulfate (3b) [34]. Of the two surfactants, 3a gives better separation of enantiomers such as DNS-amino acids, bi-β-naphthol and many pharmaceuticals containing β-hydroxylamines and barbitals.

Both amino and tartaric acids were used as chiral skeletons constituting polar headgroups. Sodium salts of both (*R*,*R*)-N-decyldiacetyltartaric acid monoamide (4) and the corresponding N-dodecyl derivative formed micelles with the ability to resolve enantiomers of N-acylated phenylethyl and naphthylethylamines and bi-β-naphthol by EKC [42].

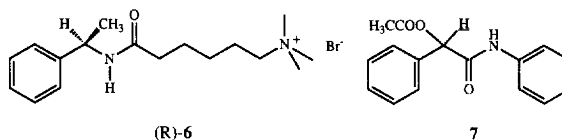
Enantiomer separation by chiral micelles is thought to occur through diastereomeric complexation in which enantiomeric solutes become intercalated among chiral functionalized surfactants into the shallow hydrophobic interior region in accordance with interaction mode (C) shown in Fig. 2.

1.2. Recognition by chiral auxiliaries in micelles

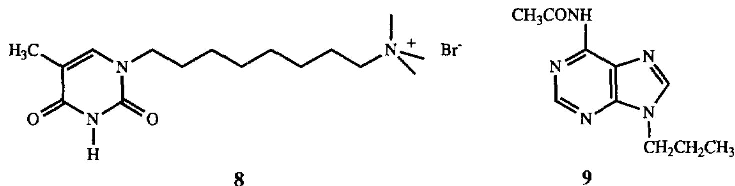
There are comicellar systems in which achiral surfactants provide only a hydrophobic environment and chiral auxiliaries possessing binding elements for substrates are solubilized in this environment. This situation is simpler than the micellization of chiral surfactants since surfactants solubilize bonding elements directly by forming a coat around them.

Binding affinity in enantiomer recognition with CSPs is expressed not only by hydrogen bonds but π–π stacking (or π-base–π-acid interaction), electrostatic interactions and coordination to metals [14–16]. One or more of these factors are definitely or possibly involved in diastereomeric complex formation. Metal-chelating interactions involved in ligand exchange on the center metal have been found effective for separating enantiomeric N-dansyl-amino acids when the N,N-didecyl-L-alanine–copper(II) complex solubilized into SDS micelles was used in EKC [43]. Instead of this ligand exchange chromatography, micellar-enhanced ultrafiltration with the chiral L-5-cholesterol glutamate–copper(II) complex solubilized in non-ionic surfactant micelles may be used for large scale separation [44]. This metalomicelle shows greater binding preference for D-phenylalanine compared to the L-counterpart, as indicated by ternary copper(II) complex formation.

With the comicelles of an enantiomeric trimethylammonium-terminated surfactant containing an amide unit (6) and SDS, recognition could be made of N-phenyl-O-acetylphenyllactamide (7) by proton NMR [45]. The resonance of hydrogen attached to an asymmmetric carbon of enantiomeric 7 are split into two peaks for each enantiomer. Using cetyltrimethylammonium bromide (CTAB) as cosurfactant, splitting of the NMR resonance ceased to occur. Greater water penetration into the micellar interior compared to SDS–6 micelles may have been the reason for this, which would be indication of a downfield shift of hydrogen resonance of 7 greater for CTAB–6 than SDS–6 micelles. Chiral recognition would thus appear to come about through hydrogen bonding between a chiral surfactant and the enantiomer present in the hydrophobic environment.



(R)-**6** **7**

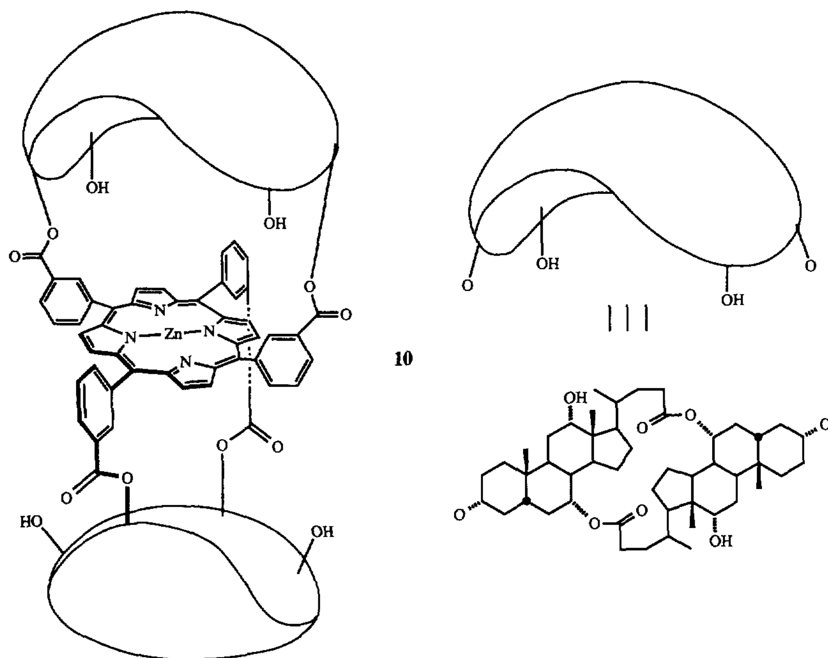


A model in which micelle formation exclude bulk water from hydrogen bonding sites of surfactants, thus providing a microenvironment suitable for binding, was validated by the base-pairing of surfactants comprising the (thyminyloctyl)ammonium group (8) and acetyladenine (9) in SDS micelles [46–48]. SDS concentration dependence on chemical shifts of protons of the thymine derivative 8 and upfield deviation with increasing SDS indicates this derivative to be incorporated in SDS micelles. A study on the binding of 9 in SDS–8 micelle using proton NMR titration indicated specific hydrogen bonding of this substrate to thymine derivative 8 to occur in SDS micelles but the binding constant to be less than in the case of polar organic solvents such as chloroform. SDS micelles may thus be considered to provide an environment comparable with polar organic solvents.

A study using SDS micelle-solubilized zinc porphyrin capped with a steroidal structure on either face (10) has shown the chiral recognition of amino acid methyl esters to be greatest when hydrogen bonding and hydrophobic interactions act in concert [49]. In the model proposed for the recognition of amino acid methyl esters, there is coordination of amino groups with zinc atoms, hydrogen bonding of ester carbonyl groups to one or more steroidal hydroxyl groups and hydrophobic interactions of amino acid side chains to steroidal non-polar roofs. Binding inside SDS micelles was found energy-wise to be essentially the same as that in methanol.

1.3. Hydrophobicity of micellar core in relation to enantiomer separation

As already indicated for 1a micelles [23,24], chiral



recognition by micelles derived from the hydrogen bonding surfactants may be possible in consideration of (1) the formation of a chiral barrier comprised of polar headgroups containing binding sites on the Stern layer, (2) hydrogen bonding of chiral elements buried in micellar interior with enantiomeric solutes and (3) differences in the extent of chiral barrier disruption due to the penetration of solutes, this being the determining factor of chiral recognition.

The separation of enantiomeric DNB-amino acid isopropyl esters in EKC obtained with 1a was found best and that by N-dodecanoyl-L-alanine (1d) least due to differences in the bulkiness of the amino acid residues of these surfactants. The lowest enantio-selectivity by 1d micelles may have been due to the lesser disruption of micellar structures owing to less steric bulkiness of 1d when amino acid derivatives with configurations opposite those of the surfactants became intercalated into micellar interior.

The degree of chiral barrier formation was assessed based on differences in aggregation of pure enantiomeric and racemic surfactants [42,50] and the CD spectrum of 1d surfactant. Enantiomeric 1a showed smaller CMC than the corresponding racemic surfactant [50]. Micelles of enantiomeric 1a thus have greater adhesive force for chiral headgroup assembly due to regular orientation and this would result in more compact packing on the micellar interface. This difference in CMC has been observed for a combination of the same types of surfactants from alanine, phenylalanine [50] and serine [41].

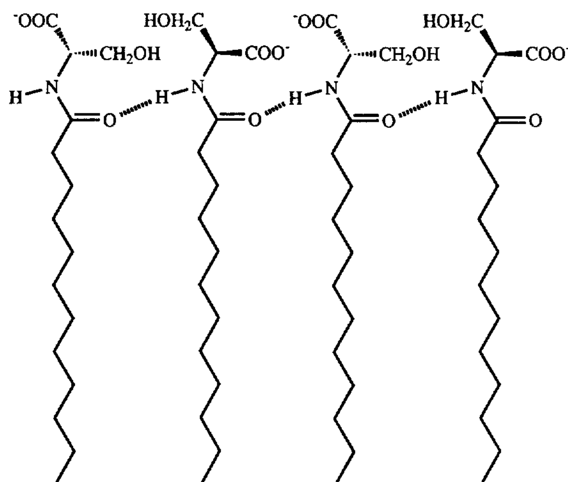


Fig. 3. Alternating tilt of the amide plane of 1b associated with the formation of NH–O=C intermolecular hydrogen bonds which cover the micellar surface.

The CD spectrum of micelles of enantiomeric N-palmitoyl- and N-stearoylserines indicated the formation of chiral assemblies of amide planes on the micellar surface as shown in Fig. 3. This would suggest alternating tilt of the amide plane associated with NH–O=C intermolecular hydrogen bond formation over the micellar surface, supported and aligned by strong hydrophobic interactions of long hydrocarbon chains. Such a hydrogen bonding network of chiral headgroups for the micellization of all N-acylamino acid surfactants may quite likely serve as an alternative adhesive force and determine the orientation of the surfactant.

Proton NMR of the micellization of 1a and 1d indicated shielding of amide units from bulk water [24]. A chemical shift in the micellar solution is the weighted average of chemical shifts of surfactants present within micelles and that of monomerically dispersed surfactants during fast association–dissociation exchange. Thus, when total surfactant concentration exceeded CMC, chemical shifts of the amide protons gradually shifted upfield and then reached virtually a plateau. This indicates amide shielding from the bulk water, making it possible for amide units to function as hydrogen bonding sites in the shallow hydrophobic region near the Stern layer. The upfield deviation for 1d was less than that for 1a throughout the region, possibly due to the greater water penetration of 1d than 1a micelles. No differences in the aggregation of enantiomeric and racemic surfactants as reflected in those of NMR chemical shifts could be found in both 1a (A. Dobashi, M. Hamada, unpublished results) and 1d surfactant [51]. The considerable similarity of micelles shown at least by NMR may possibly be due to weighted averages of rapid interconversion of conformational states of chiral headgroups and fluidity of the hydrocarbon chain by the rotation of carbon–carbon bonds.

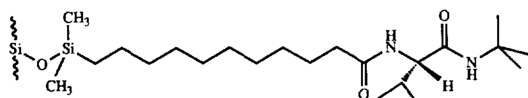
Disruption of the chiral barrier formed by anionic surfactants may likely occur in a local region of enantiomeric micelles where a enantiomeric solute becomes intercalated among hydrogen bonding networks. Thus, dilution of the chiral barrier by achiral surfactant such as SDS would result in less sepa-

ration of enantiomers [24]. Increase in SDS concentration as a cosurfactant lessened the separation of enantiomers but led to greater retention of these enantiomers or, in other words, expanded separation windows extended from retention of a solute not retained by micelles at all to that of micelles in EKC. This has been noted for many chiral micellar systems in EKC [21,26–29].

1.4. Recognition by micelles with chiral cationic surfactants

Trimethylammonium-terminated surfactants whose hydrocarbon chains contain hydrogen bonding valinediamide moieties were effective for separating enantiomers in EKC (A. Dobashi, T. Ono, M. Hamada and J. Yamaguchi, unpublished results).

Recognition in aqueous liquid chromatography (LC) is due to the hydrophobic interfacial face in which water is excluded to greater extent compared to polar or protic solvents and thus hydrogen bonding with substrates is extensive [52–55]. The interfacial face is formed by CSPs terminated with the valinediamide moieties (11) under aqueous media [55]. Chiral bonding sites are considered to be in local hydrophobic aggregates formed by both the valinediamide moiety and the decamethylene spacer separating it from the silica gel surface.



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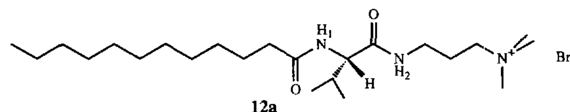
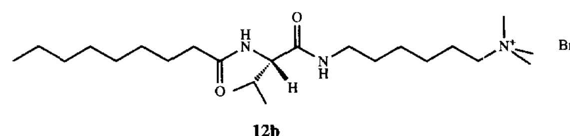
If the solid support is removed following addition of polar head groups to terminals of grafted moieties, local aggregates on the surface transform micelles hydrophobically formed without the solid support. Surfactant 12a having trimethylammonium bromide as the terminal polar head group and valinediamide as the chiral selecting group formed micelles with CMC of 1.8 m*M* in 0.1 *M* tris(hydroxymethyl)-aminomethane (Tris)–hydrochloric acid buffer (pH 7.0). A fluorescence intensity ratio of III band (λ= 383 nm) to I band (λ=373 nm), III/I ratio, of pyrene solubilized in the micelles, indicating hydrophobicity of the micellar interior [56], showed 1.10, to exceed that of dodecyltrimethylammonium bromide (DTMB) in the same buffer solution. Surfactant 12b has the quaternary ammonium and valinediamide moieties, as does 12a, but the spacer between these moieties and N-acyl group differ from those of 12a, possessing hexamethylene and N-nonanoyl group. Surfactant 12b has a longer spacer so that the valinediamide moiety should be more deeply situated within the micellar interior on postulate of a surfactant structure stretched straight. CMC measured by the pyrene fluorescence was 35 m*M* in pure water, this considerably exceeding that of 12a, and the III/I ratio of 1.03 in pure water, compared to 1.28 for 12a. Hydrophobicity of 12b micelle is thus shown to be less than that of 12a micelle.



12a



12b

Chiral micelles formed by 12a and 12b have the similar capacity to separate enantiomers of the same amino acid derivatives (substituted by benzoyl (Bz), 4-nitro-benzoyl (NB), and 3,5-dinitrobenzoyl (DNB) in each as a N-protecting group) as those separated by micelles of anionic surfactant 1a. Table 1 shows the EKC resolution of enantiomeric DNB-amino acids and corresponding isopropyl esters with 12a and 12b surfactants.

The L-enantiomer having the same configuration as the chiral surfactant was retained to a greater extent than the D enantiomer, as also noted in separation using anionic surfactant 1a. 12b was observed to have greater selectivity than 12a for DNB-amino acid isopropyl ester derivatives. The effects of N-protecting groups on the selectivity of 12a and 12b surfactants were not as clear as observed for anionic surfactants 1a, and difference in selectivity for these surfactants could not be detected for other Bz and NB derivatives. 12b micelles showed smaller capacity factors for separation of amino acid derivatives than when using 12a. The faster elution of solutes may be considered due to the

Table 1
Chiral separation of enantiomeric 3,5-dinitrobenzoyl(DNB) amino acids and corresponding isopropyl esters with cationic 12a and 12b micellar solutions by electrokinetic chromatography[a]

Solute	12a		12b	
	k'^{b}_{D}	α	k'^{b}_{D}	α
DNBAlaO-*i*-Pr	1.19	1.20	0.74	1.21
DNBValO-*i*-Pr	3.23	1.25	1.93	1.25
DNBLeuO-*i*-Pr	7.34	1.34	4.28	1.41
DNBPheO-*i*-Pr	11.3	1.16	6.75	1.22
DNBAlaOH	0.66	1.00	0.08	1.00
DNBValOH	2.04	1.08	0.58	1.10
DNBLeuOH	6.61	1.29	1.99	1.31
DNBPheOH	11.98	1.12	4.44	1.15

[a] Conditions: column, fused-silica tubing 50 cm×50 mm I.D. surfactant solution, 0.025 *M* of 12a and 0.05 *M* of 12b in 0.1 *M* tris(hydroxymethyl)aminomethane (Tris)–hydrochloric acid buffer (pH 7.0) to make micellar concentration the same as that obtained by subtracting CMC from total surfactant concentration; total applied voltage, ~ −10 kV; constant electric current, 12 μA for 12a and 16 μA for 12b migrating solutions; detection, UV at 254 nm; temperature, ambient.

[b] D-Enantiomers eluted faster than L-enantiomers.

lesser hydrophobicity of micellar interior core of 12b.

DNB-amino acids except the alanine derivative were separated by cationic but not anionic surfactants. This resolution was due to the attractive force between quaternary ammonium headgroups and solutes negatively charged by dissociation in bulk water and at least to some degree to reduction in the repulsive force between polar headgroups by the intercalation of solutes. DNB-amino acids are thus taken into micelles of 12a and 12b according to their hydrophobicity.

1.5. Hydrophobicity in cationic micelles as determined by proton NMR

Difference in the depths of two amide units of the valinediamide moiety present in a cationic surfactant can be determined by NMR. The result would indicate whether a C-terminal amide near the quaternary ammonium group is situated at a lesser depth interior than the N-terminal amide, as would appear to be the case for a surfactant structure stretched straight. This determination was made by a concentration dependence study of two amide protons of 12a as shown in Fig. 4. The degree of shielding of these amide units along with micellar formation were clarified. When the concentration exceeded CMC (6.0 m*M* in water), chemical shift of the N-terminal amide proton (H1) deviated upfield from 8.08 at 5 m*M* to 7.88 at 100 m*M*, thus showing the N-terminal amide to be shielded from water through micelle formation. The C-terminal amide proton (H2) of cationic surfactant 12a has no concentration dependence (δNH 8.33). The N-terminal amide is thus assumed situated more deeply in the micellar core than the C-terminal amide.

The distance from the polar head of the C-terminal amide of 12a is essentially the same as that of the unique N-terminal amide of anionic surfactant 1a but still the C-terminal amide situated beside the polar head is not shielded from the bulk water. This is in complete contrast to the amide of anionic surfactant 1a. The III/I ratio of the quaternary ammonium salt-type surfactant with pyrene fluorescence is generally low owing to the greater penetration of water into micellar interior compared to anionic surfactants such as SDS.

Should the difference in concentration dependence of the amide proton chemical shift of 1a and 12a reflect a different degree of water penetration into the micellar interior, micelles formed by 12a may possibly have spaces between surfactants wider than 1a,

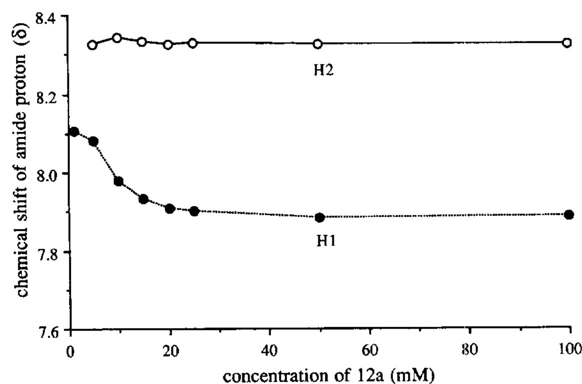


Fig. 4. Effects of 12a concentration on chemical shifts of amide protons in 10% D_2O/H_2O containing 0.2% *tert.*-butyl alcohol as the internal standard at 25°C. ● = Chemical shift of a N-terminal amide proton of 12a (H1); ○ = that of a C-terminal amide proton of 12a (H2). Terminal methyl hydrogens of dodecanoyl groups in 12a surfactant showed nearly constant chemical shifts throughout the region.

that is, be more porous, when considering the micellar structure in the Stern layer. This difference may be due to the greater steric bulkiness of trimethylammmonium head group of 12a than that of the carboxylate of 1a. ^{13}C-NMR of a quaternary ammonium-terminated surfactant with alkyl chain incorporating a carbonyl group indicated water to penetrate the micelles at least up to first seven carbon atoms of the chain [57]. Proton NMR study of 12a surfactant detected explicitly different degree of water penetration in depth toward the micellar interior.

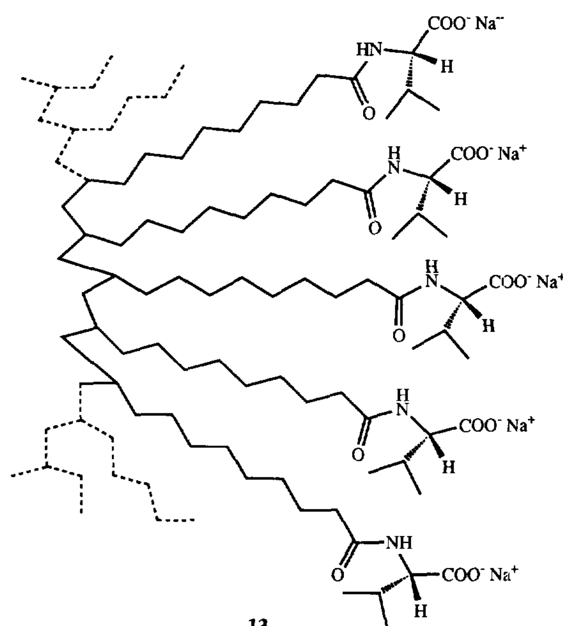
Proton NMR study indicated two amide protons of 12b to be exposed to water to a greater extent compared to those of 12a. The concentration of 12b ranged from 25 m*M* to 200 m*M* and the N-terminal amide proton shifted upfield from 8.05 at 25 m*M* to 7.94 at 200 m*M*. The chemical shift change was notably smaller than that observed for 12a. The C-terminal amide proton of 12b shifted not upfield but downfield slightly from 8.05 to 8.19. The reason for this can not be explicitly presented but the following possibility is worthy to be considered. The valinediamide moiety having polarity second to that of the polar head group in the surfactant is situated almost in the middle of the alkyl chain, thus disrupting the hydrophobic and hydrophilic balance essential for micelle formation. The aggregation number of 12a was found to be 61 in 0.1 *M* Tris–hydrochloric acid buffer solution (pH 7.0) by light scattering measurement. This aggregation number indicates the formation of the globular micelles of 12a in the same manner as with ordinal cationic surfactants such as dodecyltrimethylammonium bromide (DTMAB). In contrast, the aggregation number of 12b, 696 in the buffer solution, is much higher than that of 12a and thus 12b micelles would not likely be globular in shape.

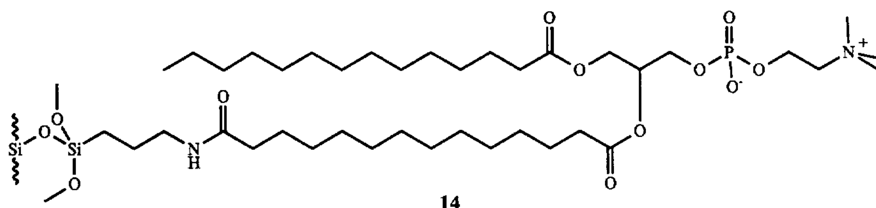
Proton NMR and pyrene fluorescence measurements of 12a and 12b surfactants finally indicated the micellar interior of 12b not to be very much shielded from water compared with 12a. 12b was however more effective, at least in part, for enantiomer separation than 12a and consequently, the chiral valinediamide moiety may be concluded a more effective binding site for solute enantiomers even in a lesser hydrophobic environment in which water penetration is greater.

1.6. Structures and hydrophobicity of micelle-like polymers

Micelles are transient aggregates formed under rapid exchange of surfactants between micellar and monomeric states in bulk water. There thus should be no a definite configuration for micelles, in considerable contrast to macrocyclic compounds possessing spatially restricted hydrophobic cavities such as chiral crown ethers [58] and cyclodextrins. Cyclodextrins and their derivatives have been shown capable of separating various enantiomers in EKC [20].

A micelle-like polymer was prepared by β- [59] and UV-irradiation of a vinyl group-terminated sodium (10-undecenoyl)-L-valinate [24,60,61], virtually the same as 1a. This polymer (13) made by covalently linking tails of surfactants has no longer CMC or micellar association–dissociation equilibrium in which surfactants are dispersed in bulk water. In UV-irradiation procedure, the average molecular weight of polymer obtained from the light scattering data was 6.93×10^4, corresponding to an aggregation number of 227. Enantiomers of bi-β-naphthol, laudanosine [59] and N-acylamino acid esters [24] were separated in EKC using the micelle-like polymers.





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The ordering of surfactants by their covalent linking may be concluded not to prevent the binding of substrates to micelles, as also indicated by comparison of poly (sodium 10-undecenoate) and sodium 10-undecenoate micelles [62]. A liquid-like core situated near the micellar center is thus not required for enantiomer binding or recognition, both of which are appeared in an ordered interfacial region formed by amino acid residues. For the amino acid derivatives, the polymer provided the lesser separation factors than the micelle of ordinary 1a surfactants. Cessation of kinetic association–dissociation of surfactants by polymerization had no effect on enhancing degree of chiral separation and the lesser selectivity of a chiral polymer may be due to spaces between surfactant monomers followed by water penetration to a greater extent to the interior core.

Poly (sodium undecenoate) undergoes conformational transition at pH 8.5 to take on a looser expanded hydrophobic structure at higher pH region [63]. This looser conformation is considered to promote separation of enantiomeric laudanosine and better resolution (R_s) in EKC using a pH10 polymer solution was noted due to a narrower peak width for each enantiomer. For certain cases of enantiomeric separation, high pH may contribute to enhance resolution factors but not probably separation factors between enantiomers. But for the amino acid derivatives, no significantly enhanced enantiomer separation was noted [24].

1.7. Surfactants immobilized on a solid support

Aqueous LC using phosphatidylcholine-bonded silica gel (14) separate selectively hydrophilic small peptides each possessing six to eight amino acid residues with or without a cysteine residue [64,65]. This chromatography in which half the lipid bilayer is immobilized on the solid support is based on hydrophobic effect in the hydrocarbon chain of phosphatidylcholine and additional electrostatic and steric interactions by its polar headgroup.

On consideration of 'mobilised' micelles in EKC and 'immobilized' micellar structures on solid supports in LC, many different surfactants should prove useful for modifying the solid support and separating substrates through application of hydrophobic and hydrophilic interactions in combination. Consequently, surfactants 12a and 12b were chemically bonded on silica gel to produce a hydrophobic interfacial phase (A. Dobashi and J. Ono, unpublished results). The grafted moieties must stretch owing to polar headgroups associated with bulk water and hydrocarbon tails anchored on the support. In this situation, difference in the depth of valinediamide moiety between 12a and 12b was observed explicitly for enantiomer separation; indicating 12b-bonded silica to provide greater separation of enantiomeric amino acid derivatives than 12a-bonded silica.

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