Chiral Recognition of Thiaheterohelicenes by Alkyl β-D-Pyranoside Micelles. Influence of Extension of Helix

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Chiral recognition of alkyl β-D-pyranoside micelles toward [7] and [5] heterohelicenes possessing helical structures was investigated by ^{1}H-NMR and CD (circular dichroism) spectroscopy. In dodecyl maltopyranoside micellar solution, *P* and *M* enantiomers of tetrathia[7]heterohelicene (7TH), which have rigid and stable helixes, manifested different chemical shifts in their ^{1}H-NMR spectra due to differences in the diastereomeric interactions, implying that the micelles undergo stronger recognition toward the *M* enantiomer than the *P* enantiomer. On the other hand, in octyl glucopyranoside micellar solution, trithia[5]heterohelicene (5TH) and two kinds of its derivatives which are rapidly equilibrated between the enantiomers in solution, gave no distinctly resolved ^{1}H-NMR peaks for either enantiomer even at a lowered temperature. However, these racemic [5]heterohelicenes in the micelles did develop induced CD absorptions owing to a displacement of the equilibrium, suggesting from the signs of their Cotton effects that the micelles prefer the *M* enantiomer to its antipode in conformity with the ^{1}H-NMR results of 7TH.

Key words chiral recognition; alkyl β-D-pyranoside; thiaheterohelicene; micellar effect

Chiral recognition can occur in artificial chiral environments such as membranes,[1] liquid crystals[2] and micelles.[3] This fact has attracted attention because it follows the model of chiral recognition in biological receptors. In particular, it is intriguing that chiral micelles, despite their intrinsically high fluidity, have the ability to discriminate between enantiomers of an incorporated racemic guest by way of differences in diastereomeric interactions. So far, such interactions in micelles have been mainly sustained by hydrogen bonding with guest molecules.[4] In previous paper[5] we investigated chiral discrimination by alkyl β-D-pyranoside micelles toward racemic thiaheterohelicenes as guest molecules, in which discrimination was done by hydrophobic interactions.

Thiaheterohelicenes are comprised of alternate thiophene and benzene rings as illustrated in Chart 1. Tetrathia[7]heterohelicene (7TH)[6] affords a stable enantiomer with either a right-handed (*P*) or a left-handed (*M*) helix because of a skeletal overlap between the terminal thiophene rings. Neither enantiomer undergoes racemization in solution at room temperature,[7] which provides a huge molecular ellipticity because of the wide-conjugated dissymmetric chromophore of 7TH.[8] Trithia[5]heterohelicene (5TH),[9] however, readily undergoes inversion of the helix in solution. This inversion is caused by the labile helical structure of 5TH which is, in turn, brought about only by weak repulsion between the hydrogens attached to the end thiophene rings.[10] We recently have reported that racemic 5HM (an alcoholic derivative of 5TH) was totally converted into a *P* enantiomer upon uptake by serum albumin of several species in aqueous solutions. This finding was determined by the circular dichroism (CD) measurements.[11] In contrast, incorporation of racemic 5HM or 5HA (a carboxylic derivative of 5TH) into aqueous alkyl β-D-pyranoside micelles displaced the equilibrium between *P* and *M* enantiomers of the helix to the *M* side, exhibiting induced CD (ICD).[5]

Octyl β-D-glucopyranoside (C8G) and dodecyl β-D-maltopyranoside (C12M) are known as powerful detergents for solubilization of lipids and organic substances.[12] It has been reported that the micelles formed by these surfactants associate with some sorts of pigments such as cresol red[13] and bilirubin,[14] showing the ICD in some cases. In the present paper we describe the behavior of 7TH and [5]heterohelicenes (5TH, 5HM, 5HA) in the micelles as observed by NMR spectroscopy, and compare these results of the CD studies. We then discuss the influence of extension of the helical structure.

Experimental

Reagents C8G (Dojin) and C12M (Dojin or Sigma) were used without further purification. 7TH, 5TH[15] and 5HM[11b] were prepared according to the procedures of our previous papers. [5]Thiaheterohelicene-2-carboxylic acid (5HA): An ethanolic solution of [5]thiaheterohelicene-2-carboaldehyde (0.32 g (1.0 mmol), 400 ml) was slowly added while stirring vigorously to a suspension solution which was made of an aqueous NaOH solution (0.16 g (4.0 mmol), 200 ml) and an $AgNO_3$ solution (0.34 g (2.0 mmol), 200 ml). After the mixture was stirred for 1 h at r.t., the resulting gray precipitate was filtered off. The filtrate was acidified with a concentrated HCl solution to pH=4, then was allowed to stand overnight. The solution was evaporated and concentrated under reduced pressure to *ca.* 200 ml. The resulting precipitate of 5HA was collected (0.32 g, 94.2%). *Anal.* Calcd for $C_{17}H_8O_2S_3$: C, 59.98; H, 2.37%. Found: C, 59.76; H, 2.75%.

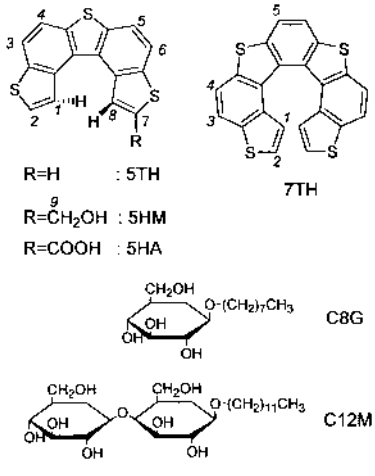

Chart 1

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Instruments CD spectra were measured on a JASCO J720WI spectropolarimeter. ^{1}H-NMR spectra were recorded on JEOL GX270FT (270.05 MHz) and A500 (500.00 MHz) NMR spectrometers equipped with a temperature variation instrument of JEOL NM-AVT3. Chemical Shifts were referenced to tetramethylsilane (TMS) in $CDCl_3$ and dioxane-d_8 solutions, and dioxane protons (4.73 ppm) in micellar solutions. UV measurements were performed with a Shimadzu UV2200A spectrophotometer.

Incorporation of Heterohelicenes into the Micelles The usual solutions for UV and CD measurements were prepared by addition of a dioxane stock solution of heterohelicene (1.0×10^{-2} mol/l, 5—50 μl) to an aqueous micellar solution (1.0×10^{-2} mol/l, 5 ml) with stirring. By that procedure, 0.1—1.0% dioxane was contained in the mixture solution, but this amount of dioxane did not affect the apparent stability of the micelles.

Results and Discussion

Thiaheterohelicenes in the present study were practically insoluble in water, only 5HA being soluble in alkaline water. However, all heterohelicenes became soluble to some extent in the aqueous surfactant solution with concentrations over the critical micellar concentration (cmc).[16]

Incorporation of 7TH into the Micelles A dioxane solution of (*M*)7TH (5 μl, 4×10^{-3} mol/l) was added to an aqueous solution of maltoside micelles of C12M (5 ml, 6×10^{-2} mol/l) while stirring at room temperature. The mixture became a slightly opaque solution, showing an intense CD spectrum of Fig. 1-b. The transparency of the solution gradually increased with time and became complete in *ca.* 50 h. This time-course provided alterations in the CD spectrum of the solution (Fig. 1-b—e). The shape of the Fig. 1-b seems rather broad and is similar well to a spectrum of solid (*M*)7TH as measured in KBr disk (Fig. 1-a).[17] In contrast, the absorptions of the transparent solution (Fig. 1-e) were fairly sharp and manifested hypsochromic shifts. The shape and sharpness of the peaks and the troughs in Fig. 1-e are in accord with those of (*M*)7TH in dioxane. The hypsochromic shifts and the intensities of the absorptions in that spectrum suggest that the (*M*)7TH molecule is located in the hydrophobic environment of the micelles. Therefore, these spectral alterations with time may be explicable in terms of aggregation and dispersion of (*M*)7TH. Initially, (*M*)7TH existed in near the surface of the micelles as molecular aggregates, which leads us to suppose a situation similar to 1,3-bis(1-pyrenyl)propane and 5,10,15,20-tetraphenylporphyrin solubilized in the micelles.[14] Thereafter, (*M*)7TH was allowed to move slowly and to localize in the inner hydrophobic portion of the micelles in the dispersion to the individual molecule. This reasoning for the alterations in the existence states of (*M*)7TH may be substantiated by the presence of several isodichroic points observed in the spectra of Fig. 1-b—e).

The (*M*)7TH solution in the glucoside micelles of C8G was prepared using the same procedures as above. In this case, however, a transparent solution was immediately obtained, and showed a CD spectrum very similar to Fig. 1-e. The difference in the behavior of (*M*)7TH between the maltoside and the glucoside micelles may be attributed to the lower hydrophilicity of the glucoside group as compared to the maltoside group as pointed out in the literature.[18] Thus, in the case of C8G, the (*M*)7TH molecules can promptly enter the inner part of the micelles. When (*P*)7TH was used in place of (*M*)7TH for both micelles, almost the same results were obtained except for the opposite Cotton effects of the CD absorptions, which indicated a small difference in the magnitude of diastereomeric interactions of the micelles with (*P*) or (*M*)7TH. This small difference is also sustained by the fact that solution of racemic 7TH in both micelles gave only very weak CD absorptions. Therefore, it is supposed that the CD measurement is not most efficient method for detecting the difference in the diastereomeric interactions between chiral 7TH and the alkyl β-D-pyranoside micelle systems.

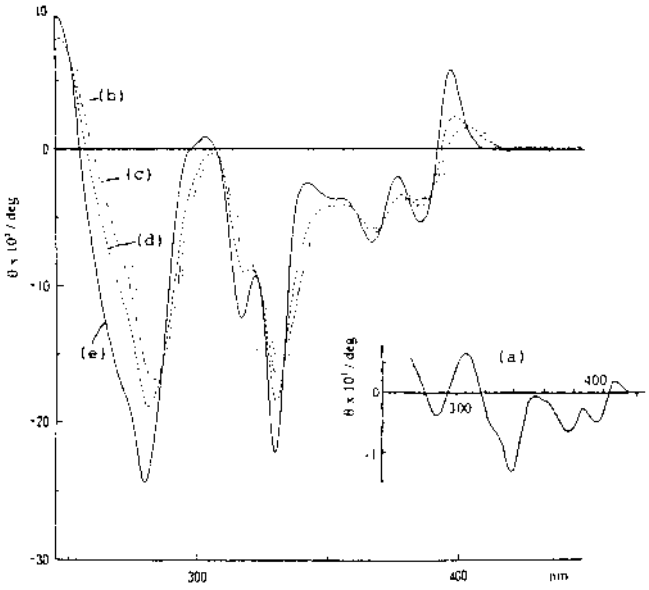

Fig. 1. CD Spectra of (*M*)-7TH

(a) in KBr disk, (b)—(e) time-course in C12M micelles at 25 °C, b; 15 min, c; 60 min d; 135 min, and e; 21.5 h. [C12M]=7.50×10^{-3} mol/l, [(*M*)-7TH]=4.36×10^{-5} mol/l.

^{1}H-NMR of 7TH in the C12M Micelles Since the 7TH molecule possesses a C2 axis perpendicular to a molecular axis, a simple ^{1}H-NMR spectrum composed of two sorts of quartet (*H1*, *H2*, and *H3*, *H4*) and one singlet (*H5*), was obtained for the racemic 7TH in chloroform (Fig. 2-a). The quartet peaks at the upper field are assigned to *H1* (6.75 ppm) and *H2* (6.91 ppm) because of an increasing ring current effect by the overlapping thiophene ring; *H1* shows the largest upfield shift due to its location right over the other terminal ring. The C12M micellar solution containing the enantiomer of 7TH, however, manifested different chemical shifts for (*P*) and (*M*)7TH, with only the *H1* protons showing almost unchanged chemical shifts. Moreover, the racemic 7TH in the micelles demonstrated the completely superimposable spectrum of two spectra for the *P* and *M* enantiomers (Figs. 2-b—d). The chemical shifts of the heterohelicenes incorporated into the micelles are tabulated in Table 1 together with those in deuterated organic solvents.

When the 7TH molecules were incorporated into the micelles, their chemical shifts were spread out over a wider magnetic field than those observed in $CDCl_3$. This may be explicable in terms of an increase in restricted motions of the 7TH molecules in the micelles. The resonance of the *H2* proton of the *M* enantiomer is found at the upper field by 0.06 ppm more than that of the *P* enantiomer, implying an increasing proximity of the proton to the other end thiophene ring. Furthermore, the central *H5* proton shows a downfield shift (0.09 ppm) for the *M* enantiomer, presumably because of an increase in an intermolecular compression effect[19] by a closer access to the alkyl chain moieties of the micelles.

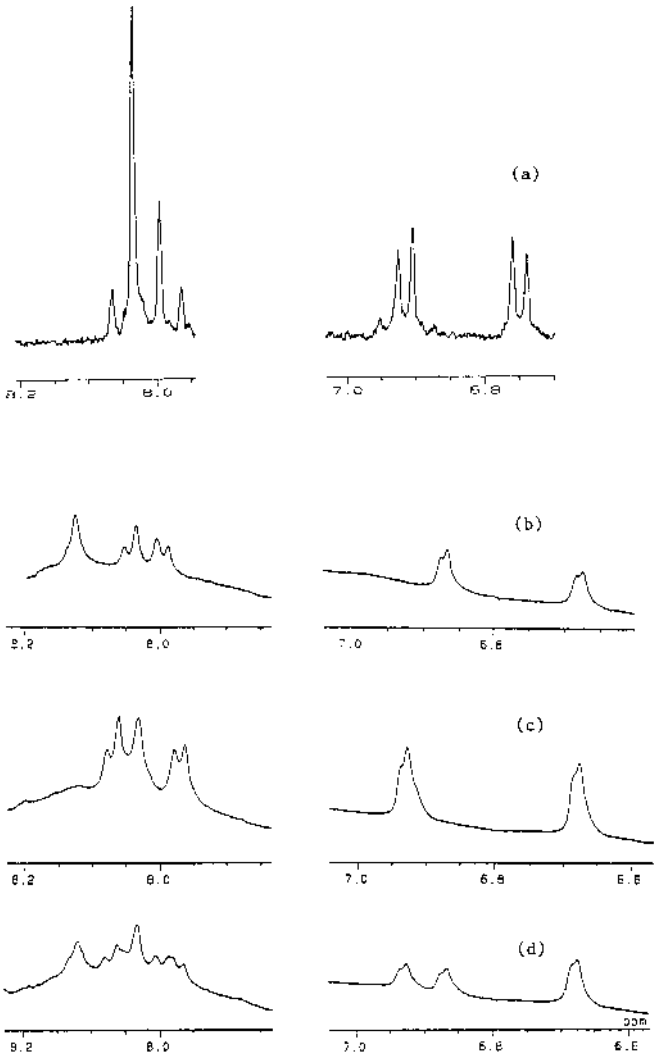

Fig. 2. ^{1}H-NMR Spectra of 7TH, (a) in $CDCl_3$, (b) (*P*) Enantiomer, (c) (*M*) Enantiomer and (d) Racemate in C12M Micelles

[C12M]=0.2 mol/l, [(*M*)7TH]=1.45×10^{-4} mol/l, [(*P*)7TH]=3.75×10^{-4} mol/l, [racemic-7TH]=2.67×10^{-4} mol/l.

These facts may suggest the stronger interaction of the micelles with the *M* enantiomer than the *P* enantiomer because the chemical shift variations of the *M* enantiomer protons are found to be somewhat larger than those of the *P* enantiomer protons. In other words, it seems noteworthy that the C12M micelles recognize the more profound chirality of the *M* enantiomer of 7TH than that of the *P* enantiomer, though the differences in the structures of the micelles containing the *P* or *M* enantiomer remain uncertain as yet. As for C8G, 7TH could not be dissolved sufficiently in that micellar solution so as to obtain the resolved peaks in a 500 MHz ^{1}H-NMR spectrum.

Incorporation of 5TH, 5HM and 5HA into the Micelles Although [5]heterohelicenes (5TH, 5HM, 5HA) undergo a rapid interconversion between enantiomers in organic solvents as stated above, incorporation of them into the β-D-maltoside micelles shifts the following equilibrium to the right, driven by the differences in diastereomeric interaction between the chiral center of the surfactants and the helical moiety of racemic [5]heterohelicene.

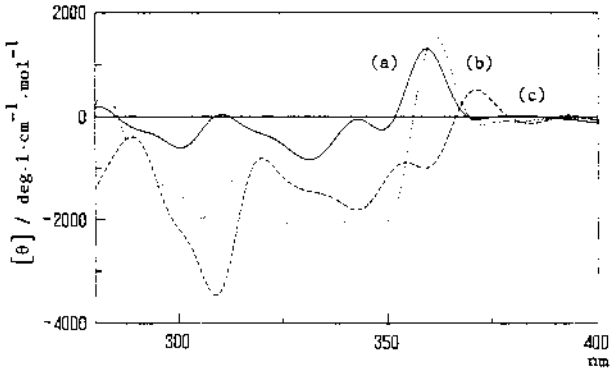

Fig. 3. CD Spectra of Trithia[5]hetrohelicenes

(a) 5TH in C12M micelles. [5TH]=3.86×10^{-4} mol/l, [C12M]=0.20 mol/l. (b) 5HM in C12M micelles. [5HM]=4.96×10^{-5} mol/l, [C12M]=7.46×10^{-4} mol/l. (c) 5HA in C8G micelles. [5HA]=2.73×10^{-5} mol/l, [C8G]=6.00×10^{-4} mol/l.

(*P*)[5]heterohelicene-C12M $\leftrightarrows$ (*M*)[5]heterohelicene-C12M

Consequently, the [5]heterohelicene molecules manifested the induced CD (ICD) due to the resulting enantiomeric excess, as illustrated in Fig. 3. The ICDs of three kinds of [5]heterohelicenes are similar in their shapes and Cotton effects to one another, despite the fact that the ICD of 5HA appears in the longer wavelength regions. In any event, it is understandable that all of these [5]heterohelicenes take an *M* enantiomeric excess in the micelles in every case, in spite of the differences in their substituents and the micelles used. However, the chiral discrimination energies of the micelles toward enantiomers of [5]heterohelicenes have been proved to be small, *i.e.* 1.5—2.7 kJ mol^{-1}, as estimated in our preceding report.[11]

This propensity of the micelles to more strongly interact with the *M* enantiomer of [5]heterohelicenes than the *P* enantiomer, is consistent with the case of 7TH in the ^{1}H-NMR study. Thus, it may be presumed that upon incorporation into the micelles, the helical moieties of 7TH and [5]heterohelicenes are located in similar surroundings near the chiral centers of the surfactant molecules. From these results it is evident that the CD measurements serve as a sensitive and direct means of detecting chirality recognition of the micelles toward the heterohelicenes.

^{1}H-NMR of 5TH, 5HM and 5HA in the Micelles The presence of an intramolecular C2 axis like 7TH, 5TH in chloroform gave a simple ^{1}H-NMR spectrum with two sorts of quartet (Fig. 4 A)). This spectrum is also characterized by a large downfield shift of the *H1* proton (8.37 ppm) because of the repulsive forces between the closely spaced terminal hydrogens. This is in marked contrast to the large upfield shift (6.75 ppm) of the *H1* proton of 7TH as mentioned above. As for 5TH in the C12M micelles, the benzene protons showed relatively small changes in the chemical shifts, while the protons attached to the thiophene rings demonstrated the following downfield shifts: *H1*, 0.02 ppm; *H2*, 0.15 ppm in comparison with the chemical shifts in $CDCl_3$. This fact may suggest that both terminal hydrogens approach more closely to each other through an increase in planarity of the 5TH molecule, which is caused by hydrophobic interactions with the long alkyl chains of the surfactants. However, no distinctly resolved peaks for the *P* and *M* enantiomers were observed, presumably because of a faster interconver-

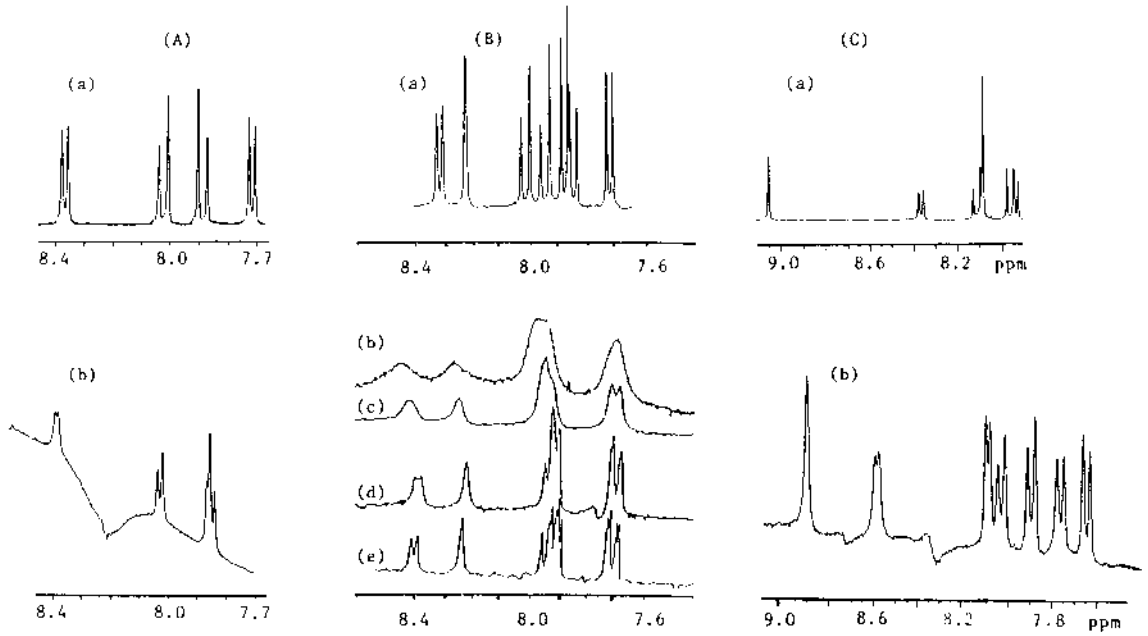

Fig. 4. ^{1}H-NMR Spectra of Trithia[5]heterohelicenes

(A) 5TH, (a) in $CDCl_3$ and (b) in C12M micelles. (B) 5HM, (a) in $CDCl_3$, and (b)—(e) in C12M micelles, (b) 5 °C, (c) 22.3 °C, (d) 44.8 °C, and (e) 64.8 °C. (C) 5HA, (a) in dioxane-d_8, (b) in C8G micelles at pD=12. [5TH]=3.86×10^{-4} mol/l, [5HM]=4.0×10^{-3} mol/l, [C12M]=0.20 mol/l. [5HA]=4.0×10^{-3} mol/l, and [C8G]=0.2 mol/l. Temperature of (A), (B)-(a) and (C) were at 23 °C.

Table 1. ^{1}H-NMR Chemical Shifts of Thiaheterohelicenes in Organic and Micelle Solutions (ppm)

	5HM		5HA		5TH		7TH		
	$CDCl_3$	C12M[a)]	Dioxane-d_8	C8G[b)]	$CDCl_3$	C12M	$CDCl_3$	C12M	
								P	*M*
H1	8.26	8.39	8.37	8.55	8.37	8.39	6.75	6.68	6.68
H2	7.68	7.90	7.93	8.05	7.72	7.87	6.91	6.93	6.87
H3, *H4*	7.84	7.72	8.07	7.73	7.89	7.85	7.98	7.98	8.00
	7.90	7.92	8.10	7.85	8.02	8.03	8.05	8.08	8.05
H5	7.81	7.71	7.96	7.61			8.03	8.04	8.13
H6	7.99	7.95	8.11	7.99					
H8	8.16	8.23	9.06	8.87					
H9	5.06	5.04							

a) These values were measured at 65 °C, and others were at 23 °C. *b*) These values were measured at pH 12, and others in the micelles were at neutral aqueous solution.

sion in the *P*/*M* equilibrium in the micelles than on a ^{1}H-NMR time scale.

Then, in order to obtain the separated peaks for both antipodes in the micelles, variable temperature measurements were carried out by using 5HM, which has the best solubility of the three in the micellar solution. The spectrum of 5HM in $CDCl_3$ consists of the peaks of three kinds of quartet and one singlet in the resonance region of aromatic protons (Fig. 4B-a). In the micellar solution, all the peaks showed broadened even at 23 °C. At a lowered temperature (5 °C), the peaks broadened further though some downfield shifts were recognized. On the other hand, the measurements at an elevated temperature (50 °C) afforded the well-resolved peaks which reasonably corresponded to the peaks observed in $CDCl_3$. The behavior of the 5HM protons in the micelles at variable temperatures may be explained in terms of an increasing constraint on their motions with a lowering of temperature. This was brought about by the formation of the hydrogen bonding between the hydroxy group of 5HM and the hydrophilic groups of the surfactants. Because of peaks broadening, no distinct peaks for either enantiomer could be obtained. Furthermore, with the change of temperature the chemical shifts of the *H1* and *H8* protons attached to the terminal thiophene rings, fluctuated more widely than did the other benzene protons. This may be attributed to an increase in the intramolecular van der Waals compression between the overcrowded hydrogen atoms on the end rings with a lowering of temperature.

On incorporation of 5HA into the C8G micelles, an alkaline condition (pH=12) was needed for dissolving it, where a carboxyl group was expected to be ionized. The ^{1}H-NMR peaks were characteristically much sharper than those of 5HM. This may suggest that the 5HA molecules are located in the vicinity of the micellar surface and can move into and out of the micelles with some extent of the freedom of motion. The chemical shifts of the 5HA protons in the micellar solution demonstrated large variations, when compared with those in dioxane: The *H1* and *H2* protons of the end thio-

phene ring showed the downfield shifts, whereas all the other protons of the aromatic rings exhibited the upfield shifts.

Although three kinds of [5]heterohelicenes, 5TH, 5HM and 5HA, did not provide the distinct resonance peaks for *P* and *M* enantiomers in the ^{1}H-NMR spectra, the distinguishing tendency of the chemical shifts in the micelles was observed in comparison with the chemical shifts in organic solvents; that is, the pronounced downfield shifts of the *H1* and *H2* protons attached to the terminal thiophene rings and the upfield shifts of the protons on the benzene rings. This tendency is reasonably explained by the micellar effect, in which the increasing hydrophobicity of the interaction of the heterohelicenes with the long alkyl chains of the surfactants brings about the upfield shifts,[20] and an increase of the intramolecular van der Waals compression effects between the terminal rings causes the downfield shifts of the end protons.

In summary, for the 7TH molecules which afford the stable enantiomers in solution at ordinary temperature, ^{1}H-NMR measurements were proved to be a more effective means of detecting chiral discrimination exerted by the chiral micelles than CD measurements. In contrast, for the [5]heterohelicene molecules which undergo a rapid racemization in solution, ^{1}H-NMR was not so good method as to trace the chiral discrimination, presumably because the inversion of their helical moieties was faster than the ^{1}H-NMR time scale. In this case, however, CD was found to play an outstanding part, because it can detect the ICD brought about from the difference in diastereomeric interactions between the chiral micelles and racemic heterohelicenes.

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