A Novel pH-Sensitive Chiral Amphiphile: Synthesis, Aggregation, and Interaction with *β*-Cyclodextrin

Chuanzhong Wang, Qiang Gao, and Jianbin Huang*

Institute of Physical Chemistry, Peking University, Beijing 100871, P.R. China

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A novel pH-sensitive chiral amphiphile, *N*-(4-decyloxy-2- hydroxybenzylidene)-L-leucine, was synthesized. Two kinds of vesicles were found at different pH ranges, 6.5−8.0 and 11.5−12.5, and a helical structure was observed at pH = 11.0. This unique behavior can be attributed to the formation of a dimeric structure. The interaction between this amphiphile and *β*-cyclodextrin was investigated by fluorescence emission spectra, ^{1}H NMR, and molecular modeling.

1. Introduction

The research on pH-sensitive amphiphiles is a rapidly growing field in colloid science, for it can be applied to many practical fields, such as use as a template for nanoparticles[1] or DNA condensation,[2] especially in drug delivery systems (DDS).[3] A substantial subject in DDS to be considered is the design of methodology for release control of the entrapped drugs under desired conditions. Many attempts have been made by designing toward a change in the surrounding conditions, such as pH,[4] temperature,[5] and UV light.[6] Among these conditions, pH has received most attention, because the pH value around the damaged tissue is usually different than that around the normal. Up to now, some pH-sensitive vesicular systems have been prepared, for example, by using a combination of unsaturated phosphatidylethanolamines and amphiphile stabilizers.[7] In our previous studies, a pH-sensitive amphiphile, *N*-(4-decyloxy-2-hydroxybenzylidene)glycine, has been reported[8] and its special surface and aggregation behaviors have been investigated with the variation of pH value in the environment.

In this paper, a novel pH-sensitive chiral amphiphile, *N*-(4-decyloxy-2-hydroxybenzylidene)-L-leucine (abbreviated as L-C_{10}HL), has been synthesized, and it was found that its aggregation behavior is greatly dependent on the pH value of the aqueous solution. The study of chiral amphiphiles is also a popular field, as they can be used in the stereoselective synthesis and in the separation of chiral materials such as pharmaceuticals.[9,10] Many groups are working in this promising field and lots of interesting results have been obtained. In this work, two kinds of vesicular structures and one helical structure were found at different pH values, and the phenomena may be attributed to the formation of a dimeric structure in the surfactant aggregates.

β-Cyclodextrin (abbreviated as *β*-CD) is also one of the most useful compounds in DDS,[11] and it is a well-known host compound in supramolecular chemistry as well. It has a unique spatial configuration, showing a cylindrical or hollow truncated cone shape. Its cavity has a hydrophobic character, while the rims are hydrophilic. These structural features give it the property of embedding molecules that can fit into the hydrophobic cavity. This unique property can be widely applied in many technological and research fields. In this work, an inclusion phenomenon was found between our synthetic molecule L-C_{10}HL and *β*-cyclodextrin. Fluorescence emission spectra, ^{1}H NMR, and molecular modeling were carried out to investigate their interaction.

2. Experimental Section

2.1. Materials. 1-Bromodecane, 2,4-dihydroxybenzaldehyde, and L-leucine were obtained commercially. Water was distilled twice from aqueous solution of $KMnO_4$, which was prepared at more than 24 h before use.

4-Decyloxy-2-hydroxybenzaldehyde. 2,4-Dihydroxybenzaldehyde (2.76 g, 20 mmol) was dissolved in 30 mL of methanol containing 1.3 g (23 mmol) of potassium hydroxide and refluxed under an N_2 atmosphere for 1 h. Then, a methanol solution containing 6.63 g (30 mmol) of 1-bromododecane was added dropwise to this mixture and refluxed for 20 h under an N_2 atmosphere. The solution was concentrated to dryness to give a mixture as a red-brown powder. The product was purified by column chromatography (light petroleum:ethyl acetate:chloroform 30:1:5 v/v/v) to give 4.46 g (80% yield) of 4-decyloxy-2-hydroxybenzaldehyde as a light yellow solid. ^{1}H NMR ($CDCl_3$, ppm): δ 0.88 (t, CH_3, 3 H), 1.27 (m, CH_2, 14 H), 1.79 (m, CH_2, 2 H), 4.00 (t, CH_2O, 2 H), 6.41−7.43 (m, C_6H_3, 3 H), 9.70 (s, HC=O, 1 H), 11.48 (s, OH, 1 H).

***N*-(4-Decyloxy-2-hydroxybenzylidene)-L-leucine.** Potassium hydroxide, 0.20 g (3.6 mmol), and 0.47 g (3.6 mmol) of L-leucine were dissolved in 17 mL of ethanol, and then 1.00 g (3.6 mmol) of 4-decyloxy-2-hydroxybenzaldehyde was added. The solution was gently stirred for 6 h at room temperature and then was concentrated to dryness to give a sticky liquid. After it was dissolved in water, diluted HCl was added to acidify the solution. The precipitate was filtered off and thoroughly washed with acetone to give 0.7 g of L-C_{10}HL (51% yield) as a yellow solid. FT-IR (KBr pellet): 3149, 2955, 2920, 2853, 1682, 1644, 1617,

* To whom correspondence should be addressed. E-mail: JBHuang@chem.pku.edu.cn.

(1) General, S.; Thünemann A. F. *Int. J. Pharm.* **2001**, *230*, 11.
(2) Mel'nikova Y. S.; Lindman, B. *Langmuir* **2000**, *16*, 5871.
(3) Sumida, Y.; Masuyama, A.; Takasu, M.; Kida, T.; Nakatsuji, Y.; Ikeda, I.; Nojima, M. *Langmuir* **2001**, *17*, 609.
(4) Collins, D.; Litzinger, D. C.; Huang, L. *Biochim. Biophys. Acta* **1990**, *1025*, 234.
(5) Brown, P. M.; Silvius, J. R. *Biochim. Biophys. Acta* **1989**, *980*, 181.
(6) Iga, K.; Ogawa, Y.; Toguchi, H. *Pharm. Res.* **1992**, *9*, 658.
(7) Park, J. M.; Aoyama, S.; Zhang, W.; Nakatsuji, Y.; Ikeda, I. *Chem. Commun.* **2000**, 231.
(8) Wang, C.; Huang, J.; Tang, S.; Zhu, B. *Langmuir* **2001**, *17*, 6389.
(9) Boyd, B. J.; Krodkiewska, I.; Drummond, C. J.; Grieser, F. *Langmuir* **2002**, *18*, 597.
(10) Alonso, C.; Artzner, F.; Suchod, B.; Berthault, M.; Konovalov, O.; Pécaut, J.; Smilgies, D.; Renault, A. *J. Phys. Chem. B* **2001**, *105*, 12778.
(11) Merino, C.; Junquera, E.; Jiménez-Barbero, J.; Aicart, E. *Langmuir* **2000**, *16*, 1557.

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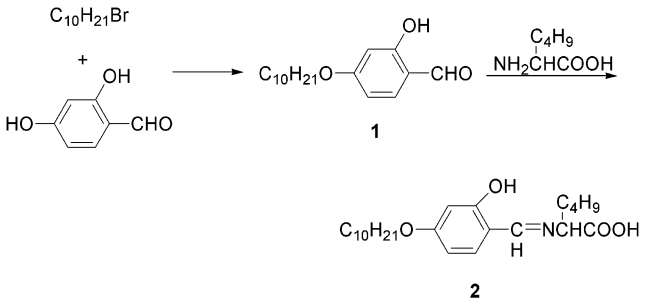
Scheme 1



1228 cm^{-1}. ^1H NMR ($CDCl_3$, ppm): 0.88 (m, CH_3, 6 H), 1.27 (m, CH_2, 18 H), 1.79 (m, CH_2, 4 H), 4.01 (t, CH_2O, 2 H), 4.05 (t, NCH, 1 H), 6.42−7.43 (m, C_6H_3, 3 H), 9.71 (s, HC═O, 1 H), and 10.33 (s, OH, 1H). UV−vis (nm, pH 12.0): 270.

2.2. Method. ^1H NMR spectra were recorded on a Varian 200 M spectrometer, with tetramethylsilane as internal reference. IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer. UV−vis spectra were recorded on a Shimadzu UV-250 spectrophotometer. Transmission electron microscopy (TEM) was carried out on a JEM-100CX II electron microscope by using the negative-staining method (uranyl acetate) or the freeze-fracture technique. Potentiometric pH titration was performed on a PHS-3B acidity meter. The fluorescence emission spectra were measured with a Hitachi F-4000 fluorescence spectrometer. Molecular modeling was carried out with the MM2 force field as implemented in Tinker3.9 software. Surface tension measurements were carried out by using the drop volume method at 30.0 ± 0.1 °C. The minimum area per amphiphile molecule (A_{min}) can be obtained from the saturation adsorption by $A_{min} = 1/(N_A\Gamma_{max})$, where N_A is Avogadro's constant and Γ_{max} is the maximum saturation adsorption.[8] The purities of the amphiphiles were examined based on a surface curve, no minimum points were found on the curves of the surface tension versus the logarithm of the surfactant concentration.

3. Results and Discussion

3.1. Synthesis and pH Titration. The synthesis route is shown in Scheme 1.

The compound **1** was prepared according to ref 12 with a little modification. After being stirred with an equimolar l-leucine, **1** was converted to **2** in 51% yield.

A pH titration was carried out at room temperature to determine the protonation constants of L-C_{10}HL. A typical titration curve is shown in Figure 1. The titration data were analyzed for equilibria 1−3 (Scheme 2). The protonation constants pK_{a1}, pK_{a2}, and pK_{a3} are 12, 9.5, and 6.5, respectively.

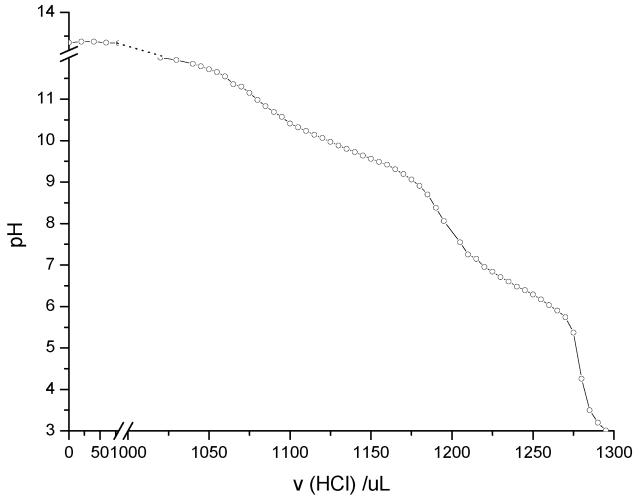


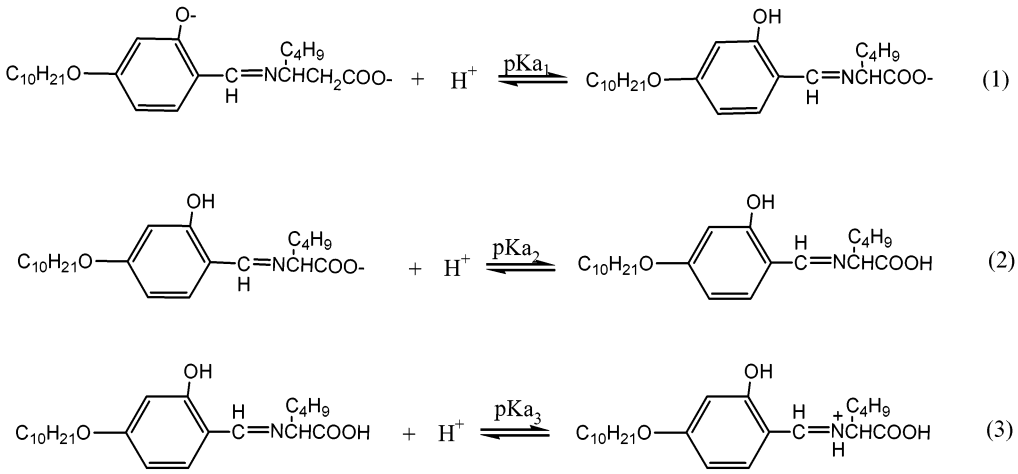
Figure 1. Typical titration curve of L-C_{10}HL: [L-C_{10}HL] = 0.01 M; [KOH] = 0.20 M; [HCl] = 0.76 M.

3.2. pH-Sensitive Aggregation Behavior. In aqueous medium, conventional fatty acids will form different types of aggregates, depending on the ionization degree of the carboxyl group. It is well-known that the kind of aggregate formed in a system depends on the value of molecular packing parameter $P = V_c/A_0l_c$,[13] where V_c and l_c are the volume and chain length of the hydrophobic group, respectively, and A_0 is the appropriate area per polar group. For vesicle formation, the proper value of P falls in the range 1/2−1. While for micelle, P is less than 1/2. In a strong alkaline solution most of carboxylic groups will be deprotonated, thus A_0 will become much larger, and P would have a small value and may be less than 1/2, as a result only micelles can be formed in the solution. With the acidification of the aqueous solution, the fatty acid molecules will be assembled into a bilayer (vesicle) at conditions where about half of the molecules are protonated.

With the addition of diluted HCl to a micellar solution of fully ionized L-C_{10}HL, we studied the aggregation behavior of L-C_{10}HL at different pH values. The morphologies of the formed aggregates at various pH values are shown Table 1.

Vesicles were found at two different pH ranges, obviously they are of different mechanisms. Global unilamellar vesicles with the size of 200−500 nm in diameter were found at a relatively low pH value, mainly from 6.5 to 8.0 (Figure 2a,b). Actually there have been many reports on

Scheme 2



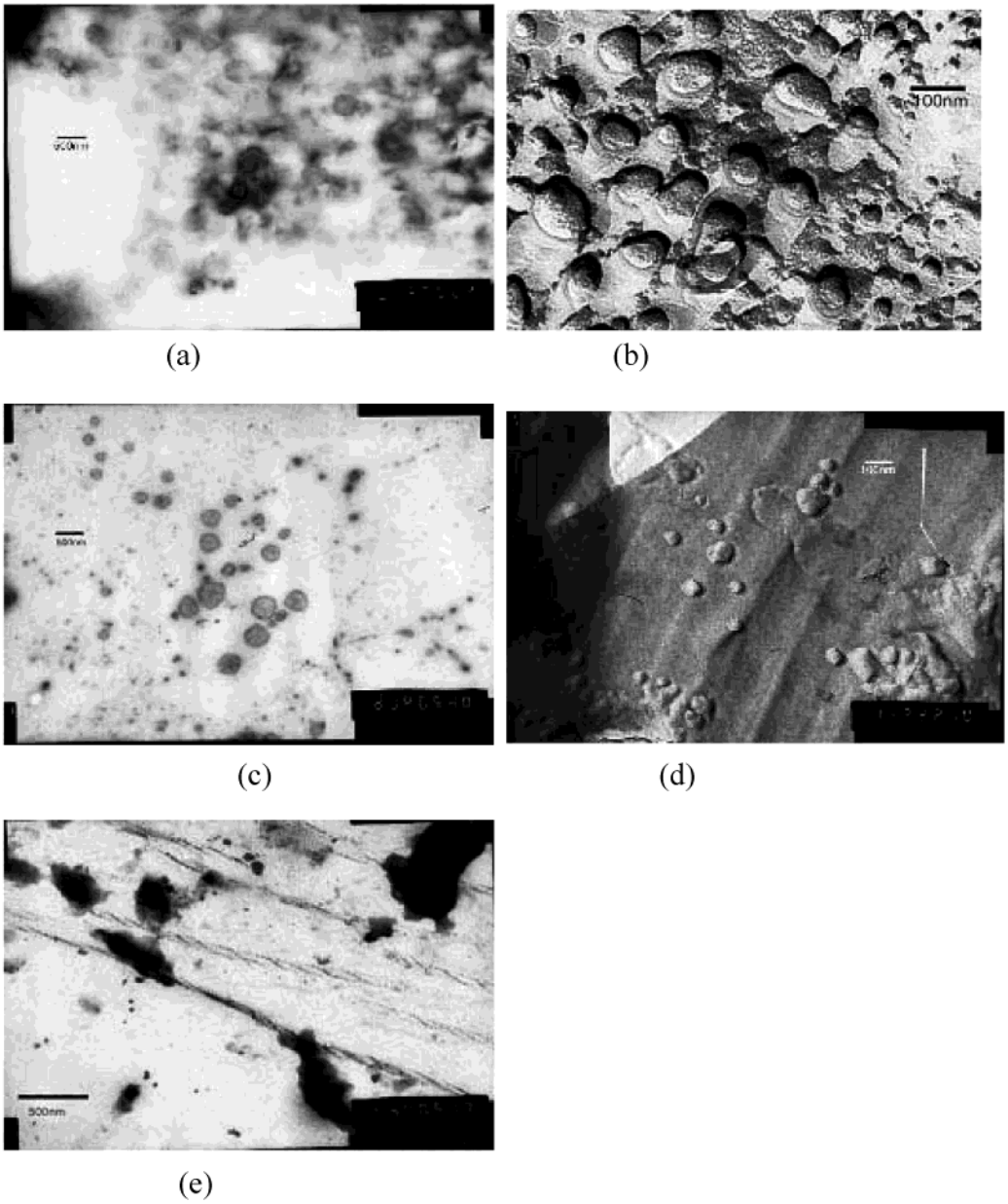


Figure 2. TEM photograph of (a) vesicular structure at pH 7.2 by the negative-staining method, (b) vesicular structure at pH 7.2 by the freeze-fracture method, (c) vesicular structure at pH 12.0 by the negative-staining method, (d) vesicular structure at pH 12.0 by the freeze-fracture method, and (e) helical structure at pH 11.0 by the negative-staining method.

Table 1. Morphologies of Aggregates Formed by L-C_{10}HL at Various pH Values[a]

pH	aggregates	pH	aggregates
13.0		9.5	
12.5	v	9.0	
12.0	v	8.5	
11.5	v	8.0	v,p
11.0	h	7.2	v,p
10.5		6.5	v,p
10.0			

[a] [L- C_{10}HL] = 0.01 M. v, vesicles; h, helical structure; p, precipitation.

the vesicle formation of conventional fatty acid in this range. Another pH range in which vesicles were found is as high as 11.5−12.5 (Figure 2c,d). For conventional fatty acids, few of the vesicular structures can be formed at such a high pH value, because almost all the carboxyl groups of the molecule are deprotonated, and the strong electrostatic force makes molecules repel each other, as a result the P factor is less than 1/2. But in our system, at pH 12.0 about half of hydroxyl groups of L-C_{10}HL are deprotonated. The negatively charged oxygen atoms in $(\text{L-C}_{10}\text{HL})^{2-}$ would attack the hydrogen atoms of the hydroxyl groups in $(\text{L-C}_{10}\text{HL})^{-}$ anions. Thus a dimeric structure would be formed (Figure 3). Obviously A_0 is greatly reduced and P will fall into the range 1/2−1, which will lead to the formation of vesicular structures. Surface tension measurements also showed that the minimum molecular area A_{min} at pH 12.0 (0.73 nm^2) is much smaller than that at pH 10.0 (0.94 nm^2). This fact implies the existence of the dimeric structure. It is worthwhile noting that the cmc, surface tension at cmc (γ_{cmc}), and the saturation adsorption (Γ_{max}) also suggest the existence of dimeric structure (Table 2). A similar phenomenon has been reported in our previous paper.[8]

(12) Claramunt, R. M.; Forfar, I.; Cabildo, P.; Lafuencente, J.; Barberá, J.; Giménez, R.; Elguero, J. *Heterocycles* **1999**, *51*, 751.
(13) Israelachvili, J. N. *Intermolecular and Surface Forces*; Academic Press: London, 1992.

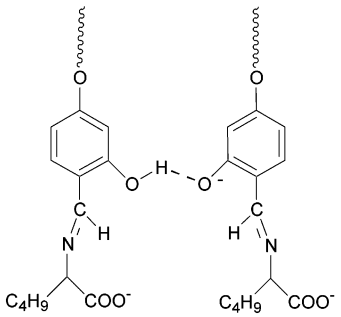


Figure 3. Dimeric structure formed by L-C_{10}HL.

Table 2. Surface Chemical Properties of L-C_{10}HL at 30.0 °C

pH	cmc, M	γ_{cmc}, mN/m	Γ_{max}, mol/m	A_{min}, nm^2
10	0.0174	31.4	1.77×10^6	0.94
12	0.0024	29.8	2.27×10^6	0.73

It is noteworthy that at pH 11.0 a helical structure of L-C_{10}HL was observed through electronic microscopy (Figure 2e). The helical superstructure is twisted ribbons with a layer thickness of 30−40 Å. This fact suggests that vesicular structures formed by chiral pH-sensitive amphiphiles can be artificially transformed into helical structures through adjusting the pH value of aqueous solution. Similar helical superstructures have been reported by other research groups.[14]

3.3. Interaction with *β*-Cyclodextrin. *β*-Cyclodextrin (abbreviated *β*-CD) has a unique property of embedding molecules that can fit into the hydrophobic cavity. In this work we found that our synthetic molecule L-C_{10}HL can form complex with *β*-CD. Fluorescence spectra were performed to measure the stoichiometry and binding constant between L-C_{10}HL and *β*-CD. The conformation of the complex was obtained by ^{1}H NMR shift titration. More detailed information about the conformation was obtained by using molecular modeling.

3.3.1. Fluorescence Emission Spectra. The curve labeled 0 in Figure 4 shows the fluorescence emission spectrum of a 10^{-5} M aqueous solution of L-C_{10}HL, characterized by a band centered at 445 nm with a low fluorescence intensity. The intensity of L-C_{10}HL in the aqueous solution increases with the addition of *β*-CD. It is well-known that the fluorescence intensity would increase with the decrease of the polarity of microenvironment around the fluorephore.[15] Thus our experimental result suggests that the polarity of microenvironment around fluorephore of L-C_{10}HL is reduced by the hydrophobic cavity of *β*-CD; i.e., the L-C_{10}HL molecule is incorporated into the cavity of *β*-CD.

According to the data of Figure 4 the stoichiometry can be obtained by[16]

$$\ln\frac{I - I_0}{I_\infty - I} = n \ln[\beta\text{-CD}] + \ln K \qquad (4)$$

where I_0 is the fluorescence intensity of L-C_{10}HL in the absence of *β*-CD, I and I_∞ are the intensities when the concentrations of *β*-CD are [*β*-CD] and in great excess. n is the stoichiometry and K is the binding constant. In our experiments, when the concentration of *β*-CD increases to above 1.37 mM, basically the fluorescence spectrum of

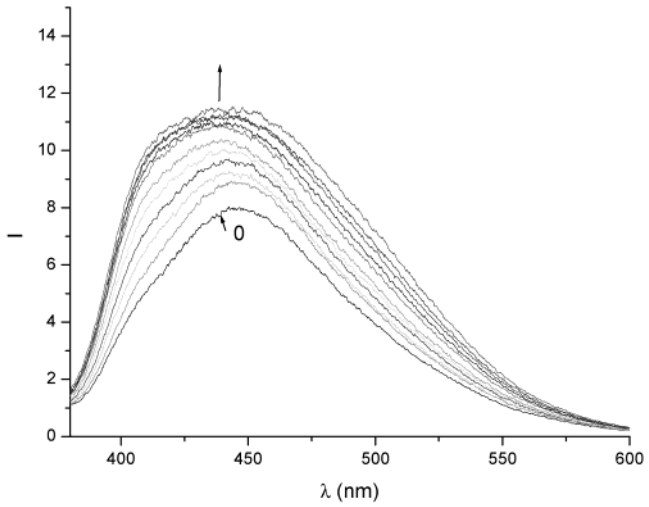


Figure 4. Fluorescence emission spectra of L-C_{10}HL at various concentrations of *β*-CD. pH = 9.2; [L-C_{10}HL] = 10^{-5} M; [*β*-CD] = 0, 2.5×10^{-5}, 5×10^{-5}, 10^{-4}, 1.5×10^{-4}, 2.0×10^{-4}, 2.75×10^{-4}, 3.5×10^{-4}, 4.5×10^{-4}, 5.75×10^{-4}, 7.75×10^{-4}, 1.37×10^{-3} M.

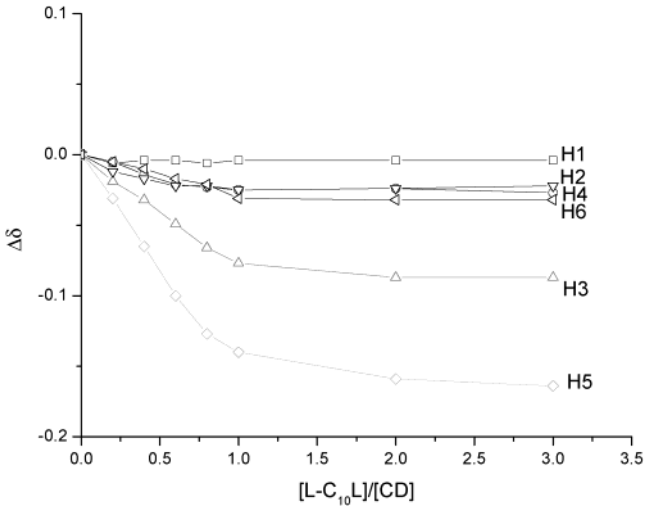


Figure 5. Chemical shifts of the *β*-CD protons versus the molar ratio [L-C_{10}HL]/[*β*-CD]. [*β*-CD] = 0.01 M at room temperature.

L-C_{10}HL will not change. Thus I_∞ can use the value of fluorescence intensity at [*β*-CD] = 1.37 mM.

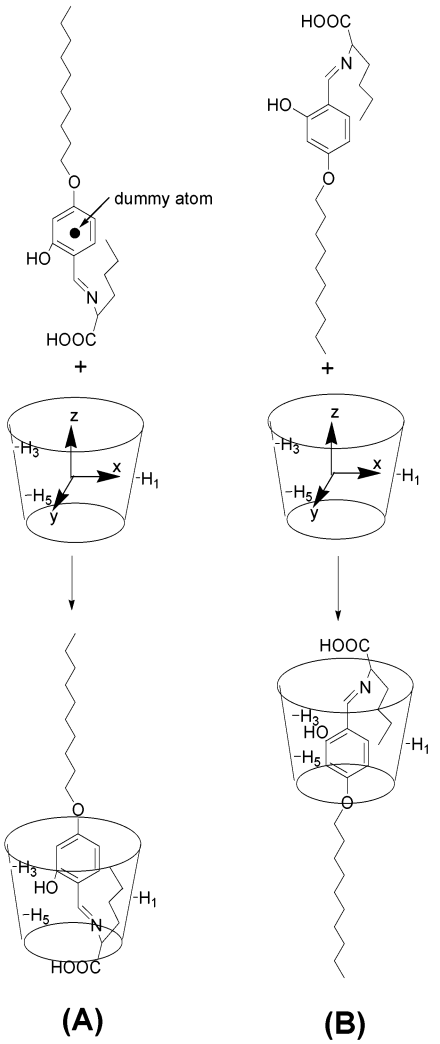
From eq 4 we can see that $\ln((I - I_0)/(I_\infty - I))$ has a linear relationship to ln[*β*-CD]. The value of n can be obtained as fitting parameters by using a linear fit. The calculated value of n is 1.028, which suggests predominantly 1:1 binding between L-C_{10}HL and *β*-CD. Some authors reported 1:2 binding in their systems;[17] however, in our system the 1:2 binding complex can be neglected. On the basis of this fact, the binding constant K can be accurately calculated by using the NLR analysis of eq 5.[18]

$$\frac{I}{I_0} = \frac{1 + K'K[\beta\text{-CD}]}{1 + K[\beta\text{-CD}]} \qquad (5)$$

where K' represents the proportionality constants connecting the intensities and concentrations. The calculated K value is 8008, which is much larger than that of

(14) Cheng, Q.; Yamamoto, M.; Stevens, R. C. *Langmuir* **2000**, *16*, 5333.
(15) Tsuchida, E.; Nishide, H.; Yuasa, M.; Hasegawa, E.; Matsushita, Y. *J. Chem. Soc., Dalton Trans.* **1984**, 1147.
(16) Wang, C. Ph.D. Thesis of Nankai University, 2000.
(17) González-Gaitano, G.; Sanz-García, T.; Tardajos, G. *Langmuir* **1999**, *15*, 7963.
(18) Merino, C.; Junquera, E.; Jiménez-Barbero, J.; Aicart, E. *Langmuir* **2000**, *16*, 1557.

Scheme 3



conventional fatty acids. This fact may be attributed to the stronger binding ability of L-C_{10}HL to β-CD.

3.3.2. ^{1}H NMR Shift Titration. The ^{1}H NMR spectra of β-CD in the presence of increasing amount of L-C_{10}HL are useful to verify the formation of the inclusion complex. Moreover, the induced chemical shifts observed upon inclusion of the guest can also help us to establish an approximate geometry for the complex. The experimental result is shown in Figure 5. Two protons, H_5 and H_3, which are located inside the cavity, show significant upfield shifts, while the resonance of the other β-CD protons show minor variations. This fact indicates that the inclusion complex of L-C_{10}HL and β-CD was formed in the solution. Moreover, the upfield of H_5 is much more than that of H_3. This fact implies a major contact with the former proton.

It is noteworthy that there is a near linear decrease in $\Delta\delta$ values up to [L-C_{10}HL]/[β-CD] = 1, which then level off beyond [L-C_{10}HL]/[β-CD] = 1. This fact implies 1:1 binding between L-C_{10}HL and β-CD, which is consistent with the conclusion drawn from fluorescence experiment.

3.3.3. Molecular Modeling. The experimental results of ^{1}H NMR gave us an outline of the conformation of

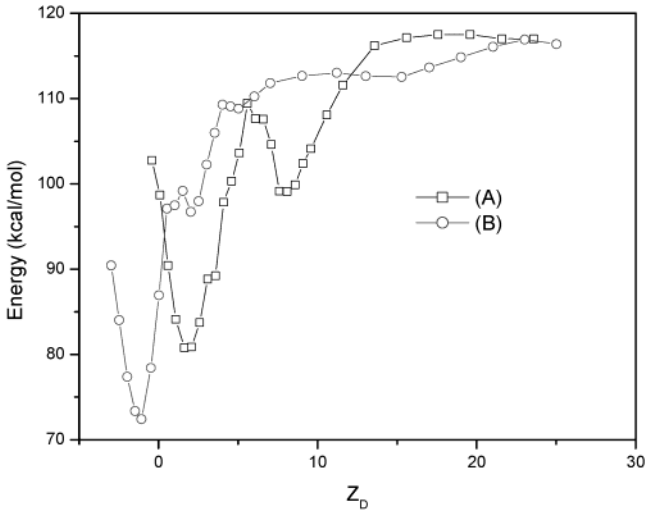


Figure 6. MM2 steric energy of our system as L-C_{10}HL is constrained to move into the interior of β-CD.

complexes. However, how the two compounds bind together is still unclear. When they bind there are two basic procedures: (A) head-in and (B) tail-in (Scheme 3). To further investigate the details, molecular modeling MM2 was employed.

An origin was placed at the center of seven H_1 atoms of a β-CD, and the z axis was aligned with the C_7 symmetry axis of β-CD. The carbon chain of an optimized L-C_{10}HL was set along the z axis. A dummy atom was placed at the center of aromatic ring of L-C_{10}HL. The distance between the dummy atom and the origin (Z_D) was fixed, and the remaining β-CD and L-C_{10}HL atoms were allowed to fully relax. Beginning with a far enough distance, the L-C_{10}HL molecule was incrementally pushed into the cavity along the z axis, and a geometry optimization followed. The calculated steric energy versus the reaction coordinate was plotted in Figure 6.

From Figure 6 we can find the minimum energies, which are 80.8 kcal/mol for procedure A and 72.4 kcal/mol for procedure B. Obviously, the latter is a fair amount smaller than the former, thus we can infer that the conformation of B is more stable than that of A.

4. Conclusions

In this work, a novel pH-sensitive amphiphile, L-C_{10}HL, was synthesized, and its protonation constants were measured by a pH titration. It is interesting that vesicles were found in two different pH ranges, pH 6.5−8.0 and 11.5−12.5, and a helical structure was observed at pH = 11.0. This unique aggregation behavior at high pH value could be attributed to the formation of a dimeric structure in the surfactant aggregate. It was also found that L-C_{10}HL could form an inclusion compound with β-cyclodextrin. Their interaction was investigated by fluorescence emission spectra, ^{1}H NMR, and molecular modeling.

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