Recognition-Induced Supramolecular Porous Nanosphere Formation from Cyclodextrin Conjugated by Cholic Acid

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A supramolecular porous nanosphere is constructed from amphiphilic cholic acid-modified cyclodextrin triggered by guest sodium 1-naphthylamino-4-sulfonate and is comprehensively characterized by nuclear magnetic resonance (NMR), light scattering, transmission electron microscopy, and gas adsorption experiments. The results obtained show that the porous nanosphere with the radius of 25−35 nm has moderate nitrogen adsorption ability. Further NMR, circular dichroism, and the fluorimetric titrations on the self-assembling behavior in aqueous solution reveal that the substituting group of the guest molecule and pH values are the key to induce the formation of the porous nanosphere.

1. Introduction

The combined processes of molecular-scale recognition and self-assembly offer a powerful route to the development of both refined supramolecular systems and, more generally, new technological devices based on controlling and analyzing interactions at the nanometer scale.[1−4] For instance, the synthesis of nanometer-sized porous spheres has found numerous applications in drug delivery/targeting and extraction,[5] nanoreactors,[6] and functional materials.[7] Therefore, the characterization of the shape, size, and properties of supramolecular nanospheres formed from synthetic receptors has been shown to be crucial for the potential application of these systems. While the general methodology employed in these studies has mainly been focused on assembling inorganic compounds or inorganic−organic composites to supramolecular porous nanospheres,[7,8] the porous nanospheres of organic aggregates have rarely been reported thus far, to the best of our knowledge. Cyclodextrins (CDs) and their derivatives are excellent building blocks to create supramolecular assemblies,[3e,9] and supramolecular nanospheres can be also constructed by using amphiphilic molecules with reasonable design.[10] However, only a few CD derivatives[11] have been aggregated into supramolecular spheres. In the present paper, we describe a new method for the preparation of porous nanospheres from an amphiphilic β-CD derivative (**1**) with cholic acid tether in the presence of sodium 1-naphthylamino-4-sulfonate (1,4-SNS). We also demonstrate that the strong host−guest inclusion interaction, the shape of the guest molecule, and pH values are the key factors to induce the formation of fullerene-shape nanospheres.

2. Experimental Section

Materials. β-CD of reagent grade was recrystallized twice from water and dried in vacuo at 95 °C for 24 h prior to use. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 days and then distilled under a reduced pressure prior to use. Dicyclohexylcarbodiimide (DCC) and cholalic acid were commercially available and used without further purification. 1,4-SNS and disodium 2,6-naphthalenedisulfonate (2,6-DNS) were purchased from Tokyo Kasei and used as received. Mono[6-*O*-(*p*-toluenesulfonyl)]-β-CD (6-OTs-β-CD) was prepared by the reaction of *p*-toluenesulfonyl chloride with β-CD in alkaline aqueous solution. Then, 6-OTs-β-CD was converted to mono(6-aminoethylamino-6-

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deoxy)-β-CD in 70% yield upon heating in excess ethylenediamine at 70 °C for 7 h.[12]

Instruments. Fluorescence spectra were recorded in a conventional quartz cell (10 × 10 × 40 mm) at 25.0 ± 0.1 °C on a JASCO FP-750 fluorescence spectrometer with the excitation and emission slits of 5 nm width. Circular dichroism (CD) and UV−vis spectra were recorded in a conventional quartz cell (light path of 10 mm) on a JASCO J-715S spectropolarimeter and a Shimadzu UV-2401PC spectrophotometer equipped with a PTC-348WI temperature controller to keep the temperature at 25.0 °C. Elemental analysis was performed on a Perkin−Elmer 2400C instrument. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded in D_2O on a Varian Mercury VX300 spectrometer. Transmission electron microscopy (TEM) experiments were performed using a Philips Tecnai G2 20 S-TWIN microscope operating at 200 kV. The samples were prepared by depositing a drop of the suspension onto a holey carbon grid.

Light Scattering Experiments. A Brookhaven Instruments BI-200SM goniometer and a BI9000AT correlator were used in static and dynamic light scattering experiments for vortically polarized light at $\lambda = 514.5$ nm with an (Innova 304) Ar^+ ion laser. The experiments were performed at 25 °C in aqueous solution by using toluene as the standard reference. The laser power was maintained at 200 mW.

When interparticle interactions and hydrodynamic corrections were neglected, the hydrodynamic radii (R_h) of nanospheres were measured in pure water at 25 °C by dynamic light scattering with various concentrations ($c = 16.7$, 12.5, 10.3, and 8.4 × 10^{-4} mol dm^{-3}). The average hydrodynamic radius was obtained with five independent experiments for each concentration. Furthermore, the molecular weight (M) and gyration radius (R_g) of the nanospheres with different concentrations were also obtained by using static light scattering. Owing to the small size of the nanospheres, we determined M and R_g using the classical equation[13]

$$\frac{Kc}{R_W} = \frac{1}{M_W}\left[1 + \frac{16\pi^2}{3\lambda^2}\langle R_g^2\rangle \sin^2\frac{\theta}{2} + ...\right] + 2A_2c \qquad (1)$$

where $K = 4\pi^2n^2(dn/dc)^2/(N_A\lambda_0^4)$, R_W is the scattering intensity, N_A is Avogadro's number, dn/dc is the refractive index increment (0.129 $cm^3\ g^{-1}$), c is the concentration, n is the refractive index of the solvent (for pure water, 1.335 20), A_2 is the second virial coefficient, and θ is the scattering angle. The Rayleigh ratios R_W were measured at a scattering angle (θ) range from 40° to 130° for each concentration of nanosphere in pure water at 25 °C. Using the Zimm plot of Kc/R_W versus $\sin^2(\theta/2)$, the molecular weight M_W and gyration radius R_g could be calculated from the intercept and slope, respectively.

Gas Adsorption Measurements. The sorption isotherm measurement of nitrogen was performed at 298 K, by using Thermo Finnigan (Sorptomatic 1990) automatic volumetric adsorption equipment. A known weight (about 200 mg) of the nanosphere sample was placed in the quartz tube, and then, prior to measurements, the sample was dried under high vacuum at 323 K for 10 h to remove the solvated water molecules. The adsorbate was dosed into the sample tube; then the change of the pressure was monitored; and the amount of adsorption was determined by a decrease in pressure at the equilibrium state.

Preparation of Mono[6-cholaminoethyleneamino-6-deoxy]-β-CD (1). To a solution of DMF (30 mL) containing 1.2 g of mono-(6-aminoethylamino-6-deoxy)-β-CD and 0.26 g of DCC was added 0.45 g of cholic acid in the presence of a small amount of 4 Å molecular sieves. The reaction mixture was stirred for 2 days in an ice bath and another 2 days at room temperature and then allowed to stand for 1 h. The precipitate was removed by filtration, and the filtrate was poured into 300 mL of acetone. The precipitate was collected and subsequently purified on a Sephadex G-25 column with water as the eluent. After the residue was dried in vacuo, a pure sample was obtained in 25% yield. ^{1}H NMR (300 MHz, D_2O, TMS) δ: 0.6−2.1 (m, 33H), 2.7−3.1 (m, 4H), 3.3−4.0 (m, 45H), 4.9−5.0 ppm (d, 7H). Elemental analysis calcd for $C_{68}H_{114}O_{38}N_2{\cdot}8H_2O$: C, 47.71; H, 7.66; N, 1.64. Found: C, 47.68; H, 7.72; N, 1.49.

Table 1. Hydrodynamic Radius (R_h), Gyration Radius (R_g), and Molecular Weight (M) of the Nanosphere of 1/1,4-SNS with Different Concentrations in Pure Water at 25 °C

concentration ($\times 10^{-4}$ mol dm^{-3})	R_h (nm)	R_g (nm)	R_g/R_h	M (g mol^{-1})
8.4	14.8	14.4	0.97	5.0×10^5
10.3	17.6	18.1	1.03	5.0×10^5
12.5	20.2	20.8	1.03	5.1×10^5
16.7	28.3	28.9	1.02	5.3×10^5

Preparation of Nanospheres. An aqueous solution of 1,4-SNS (1 mmol, 10 mL) was added to an aqueous solution of amphiphilic CD **1** (1 mmol, 10 mL) and stirred at room temperature for 2 h. The solution was dried by rotary evaporation to yield a thin film in a round-bottom flask, and the residual solvent was removed in vacuo. Then, water of 8 mL was added, and the sample solution was kept for 0.5 h at room temperature. The resulting suspension was sonicated at room temperature for 0.5 h and filtrated by using a 0.45 μm syringe filter after standing for 1 day. The filtrate was evaporated under reduced pressure, and the precipitate formed was collected by the filtration to give the nanospheres.

3. Results and Discussion

Synthesis. Mono[6-cholaminoethyleneamino-6-deoxy]-β-CD **1** was prepared by the condensation of cholic acid with mono-(6-aminoethylamino-6-deoxy)-β-CD in 25% yield. When **1** was dispersed into a thin film of water by using a sonication bath for 1 h at room temperature, no nanosphere was obtained from the solution. Interestingly, when an aqueous solution of 1,4-SNS was added to an aqueous solution of equimolar **1**, the nanosphere was formed after the obtained thin film was dispersed into water (Figure 1). From the examination of the CPK model of the **1**/1,4-SNS complex, the length of the complex is about 3 nm. The critical concentration of formation of nanospheres was about 5.0 × 10^{-4} mol dm^{-3}. Moreover, replacement of 1,4-SNS with 2,6-DNS in the experiment did not produce nanosphere.

Light Scattering, TEM, and Gas Adsorption. To confirm the size and average molecular weight of nanospheres, the light scattering experiments are performed in aqueous solution at 25 °C. As can be seen from Table 1, dynamic light scattering of the **1**/1,4-SNS aqueous solution shows that the hydrodynamic radius (R_h) of the CD aggregates is in the range of 15−30 nm and their average molecular weight (M) is 5.1×10^5 g mol^{-1}, corresponding to an average aggregation number of 283 for **1**/1,4-SNS complexes in each aggregate. On the other hand, the ratio of the gyration radius (R_g) to the hydrodynamic radius (R_h) is in the range of 0.97−1.03, which suggests a hollow or porous formation of the aggregates by the relatively incompact intermolecular association of **1**/1,4-SNS complexes.[14]

Furthermore, TEM experiments give direct evidence for the formation of supramolecular nanospheres. Figure 2 shows typical TEM images of the **1**/1,4-SNS aggregates, revealing numerous nanospheres with a radius of 25−35 nm, which is consistent with the results of the light scattering experiments. From the high-resolution TEM image (Figure 2c), we can distinctly observe the microstructure of nanospheres. There are a lot of hole structures with a diameter of 3−10 nm in the nanosphere. According to the results of the light scattering experiments, TEM

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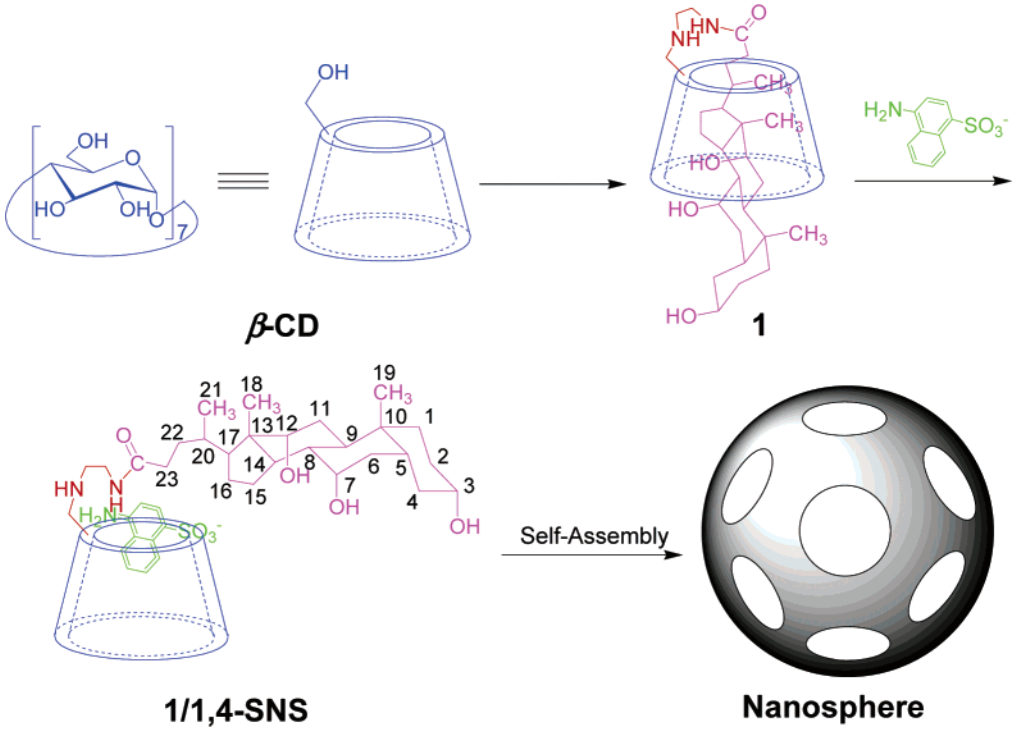

Figure 1. Suggested pathways for the formation of **1**/1,4-SNS nanospheres.

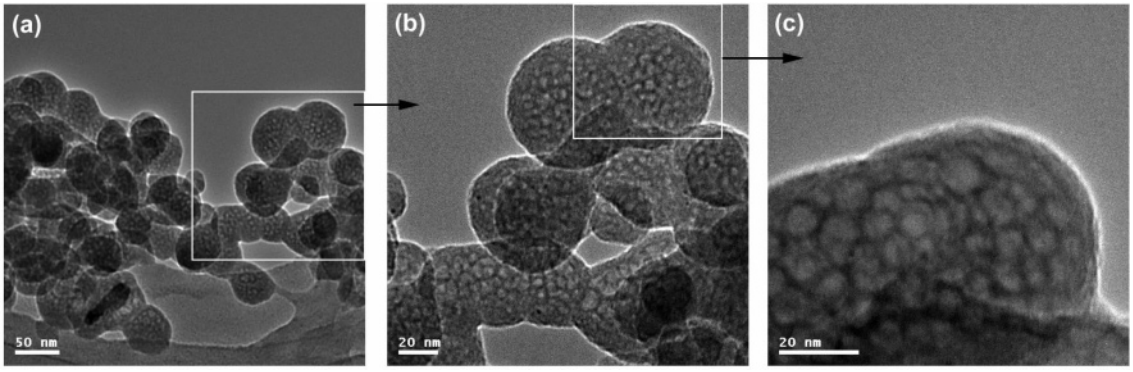

Figure 2. Typical TEM images of the nanospheres with **1**/1,4-SNS.

experiments, and some relative reports,[15−18] we could deduce a possible self-assembly mode of **1**/1,4-SNS complexes in the nanospheres, which are illustrated as in Figure 3. It is well-known that the primary driving force for the aggregation with the cholesterol groups is due to the one-dimensional stacking of the cholesterol moieties.[15] In present system, the CD complexes with 1,4-SNS should be present at the surface of the aggregation, while the hydrophobic cholic acid moieties cluster inside.

To examine the porous functionality of the nanosphere material, nitrogen adsorption isotherm was carried out at 298 K. A rapid increase in the amount of adsorbed gas was observed with an increase from the pressure of 0.95 P_0, which indicates the diffusion of guest gas molecules into the holes of nanospheres.[19] In a control experiment, modified β-CD **1** did not exhibit appreciable gas adsorption ability. The result obtained shows that the nanosphere material possesses a ca. 66 m^2/g Brunauer−Emmett−Teller (BET) surface area. Moreover, the adsorption and desorption experiments with nitrogen traced almost the same isotherms, which indicates that the porous structure is retained through this process. Because the modified β-CD **1** did not exhibit appreciable gas adsorption ability, one possible explanation for the phenomenon of the gas adsorption is that the holes of nanospheres could include the gas molecules, showing moderate gas adsorption ability. Usually, some inorganic materials or inorganic−organic hybrid materials could show higher gas adsorption ability.[20] In the present work, we determined the gas adsorption ability by using porously organic materials, for which we would like to exploit a new approach for studying the gas adsorption abilities of porously organic materials.

NMR, Induced Circular Dichroism (ICD) Spectra, and pH Effects. To investigate the role of guest 1,4-SNS for the formation of the nanospheres, we first performed a 2D NMR experiment of **1** in D_2O. As shown in Figure 4, the rotating-

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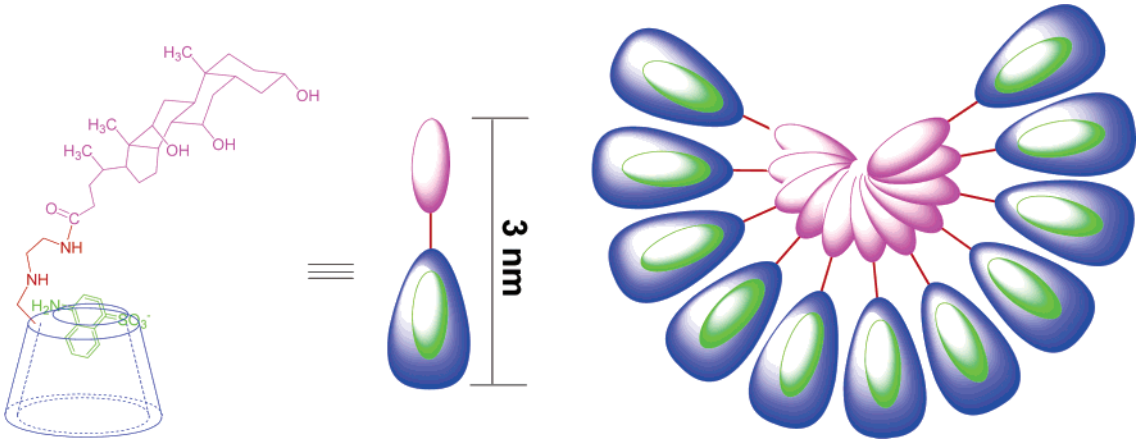

Figure 3. Probable self-assembly mode of **1**/1,4-SNS complexes in the nanospheres.

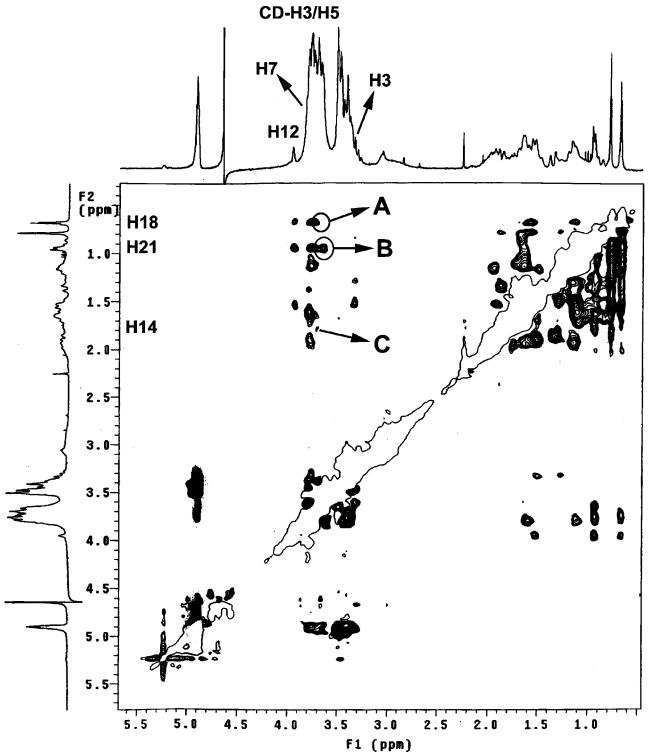

Figure 4. ROESY spectrum of **1** (1.8×10^{-3} mol dm^{-3}) in D_2O at 25.0 °C with a mixing time of 300 ms.

frame Overhauser enhancement spectroscopy (ROESY) spectrum of **1** (1.8×10^{-3} mol dm^{-3}) displayed clear nuclear Overhauser effect (NOE) cross-peaks between the H3 protons of β-CD and H18 (peaks A) and H14 (peaks C) protons of the cholic acid moiety, as well as between the H3/H5 of β-CD and H21 proton (peaks B). The results suggest that the cholic acid moiety in **1** is self-included into the hydrophobic cavity of a CD, as illustrated in Figure 1. Consequently, the self-included conformation prevents the hydrophobic segments in **1** from undergoing intermolecular association to form the nanospheres. It is documented that the complex stability constant (K_S) for the complexation of β-CD with cholate is 0.41×10^4 M^{-1} in phosphate buffer aqueous solution (pH 7.2) at 25.0 °C.[21] On the other hand, the naphthalene ring and the cavity of the β-CD give a strict size-fit relationship and relatively stronger hydrophobic interaction;[22] therefore, we selected 1,4-SNS and 2,6-DNS as molecular receptors to explore the function of the guest to the

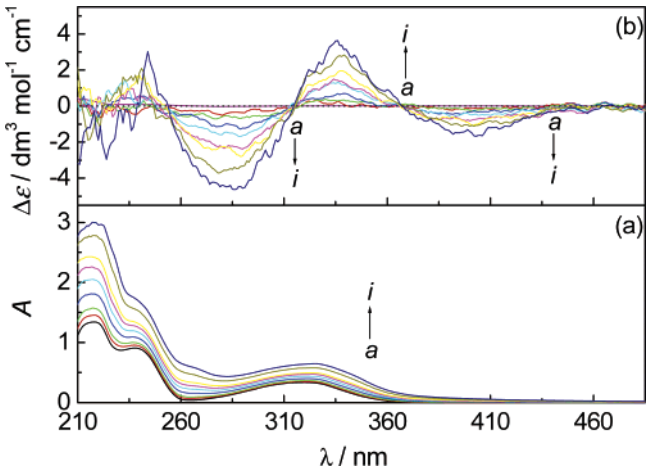

Figure 5. (a) Absorption and (b) CD spectra of 1,4-SNS (3.4×10^{-5} mol dm^{-3}) upon the addition of **1** (from *a* to *i* = 0, 0.40, 0.80, 1.60, 2.40, 3.20, 4.00, 5.60, and 7.20×10^{-4} mol dm^{-3}) in aqueous solution at 25.0 °C.

formation of nanospheres. The Job's plots confirm the formation of the 1:1 complex of **1** with 1,4-SNS/2,6-DNS in phosphate buffer aqueous solution (pH 7.2) at 25.0 °C. The complex stability constants for the stoichiometric 1:1 complexation of **1** with 1,4-SNS and 2,6-DNS are obtained by the fluorimetric titrations, and the corresponding K_S values are 4.19×10^4 and 1.04×10^5 M^{-1} in phosphate buffer aqueous solution (pH 7.2) at 25.0 °C, respectively. These values are larger than the K_S value for the complexation of β-CD with cholate; therefore, both of the guest molecules should be included into the CD cavity to change the conformation of the cholic-acid-modified β-CD **1**.

The ICD study enables us to elucidate the conformation of the interaction between the achiral chromophoric compound and the CD chiral cavity. As can be seen from Figure 5, the stepwise addition of CD **1** of up to 0.72 mM to an aqueous solution of 1,4-SNS (34 μM) causes a gradual increase of the ICD intensity and corresponding CD signals appear in ca. 238 nm (positive), 284 nm (negative), 336 nm (positive), and 401 nm (negative). Because neither 1,4-SNS nor **1** displays any appreciable CD signals in the wavelength range of 200−500 nm by itself, we can therefore deduce that 1,4-SNS is included in the cavity of CD, according to the principles of CD spectra of CD complexes.[23]

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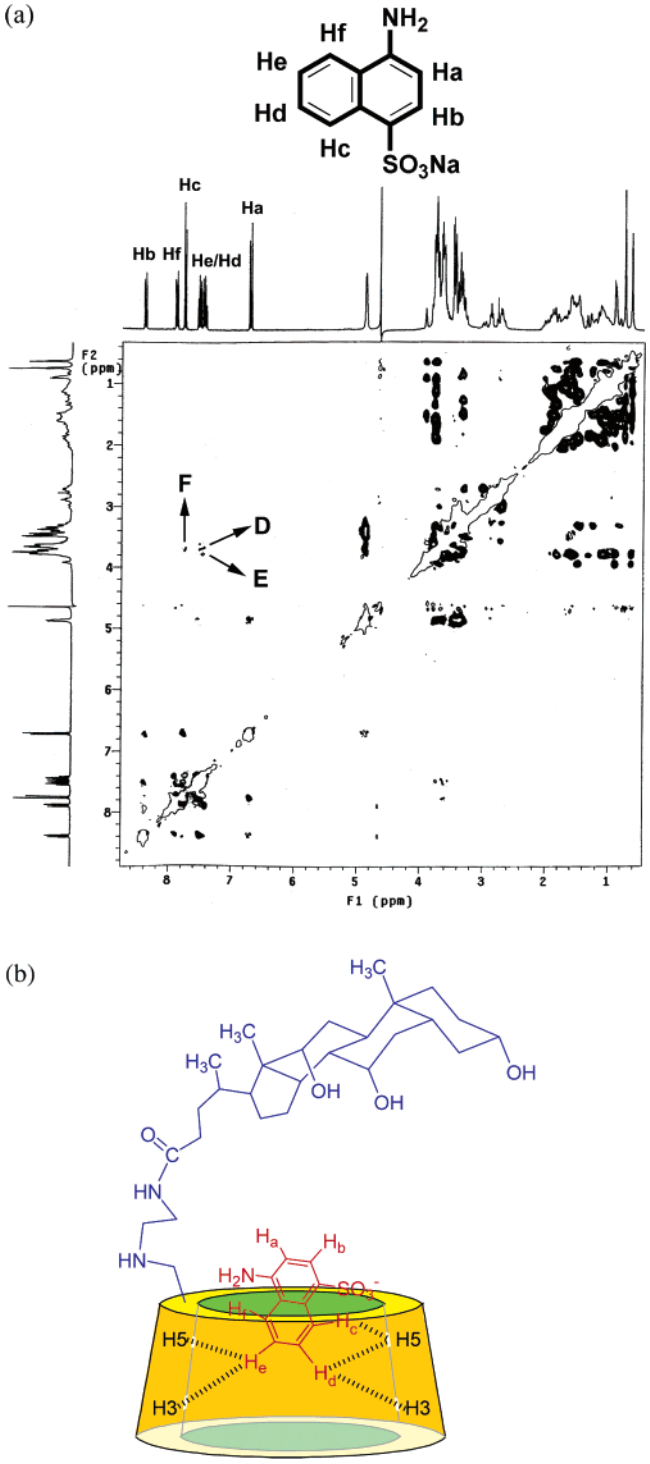

Figure 6. (a) ROESY spectrum of **1** (2.1×10^{-3} mol dm^{-3}) with 1,4-SNS (2.4×10^{-3} mol dm^{-3}) in D_2O at 25.0 °C with a mixing time of 300 ms. (b) Possible structure of **1** with 1,4-SNS in solution.

Further evidence for the conformations of complexes **1**/1,4-SNS and **1**/2,6-DNS was obtained by the 2D NMR study. The ROESY spectrum (Figure 6a) of **1** with 1,4-SNS in D_2O shows the NOE cross-peaks between the H3 of β-CD and the H_d/H_e of 1,4-SNS and the ones between the H5 of β-CD and the H_d/H_e and H_c of 1,4-SNS. However, there are no NOE cross-peaks found between the H3/H5 of β-CD and the protons of the cholic acid moiety or between protons of 1,4-SNS and the protons of the cholic acid moiety, which indicates that the cholic acid moiety must be excluded from the hydrophobic cavity of β-CD by 1,4-SNS, as illustrated in Figure 6b. Moreover, the 2D NMR experiment of **1** with 2,6-DNS in D_2O shows that not only is 2,6-DNS included in the cavity of β-CD but the cholic acid moiety is also shallowly self-included into the cavity, suggesting that there is an inclusion equilibrium between the cholic acid moiety of **1** and the 2,6-DNS molecule at the cavity of β-CD. Therefore, it is reasonable that the **1**/2,6-DNS complex cannot form the nanospheres because it could not give a good amphiphilic mode. For the **1**/1,4-SNS complex, the amino group and sulfonic acid group in 1,4-SNS are located at the primary sides of β-CD; therefore, they can prevent hydrophobic cholic acid segments in **1** from self-including into the cavity of β-CD, making the formation of nanospheres become a natural process.

To investigate the pH effects in the formation of nanospheres, we performed the self-assembly experiments of the **1**/1,4-SNS complex under the different pH values (pH = 2.2, 6.2, 7.2, and 10.0). The obtained results indicated that the nanospheres could be formed in pH 6.2, 7.2, and 10.0, while it could not be formed in pH 2.2. Furthermore, the binding abilities of **1** with 1,4-SNS are also determined with different pH values by the fluorimetric titrations. The corresponding K_S values for the complexation of **1** with 1,4-SNS are as follows: the spectral changes are too weak to calculate the K_S value for pH 2.2; 1.98×10^4 M^{-1} for pH 6.2; 4.19×10^4 M^{-1} for pH 7.2; and 5.89×10^4 M^{-1} for pH 10.0. When the pH value was 2.2, the amino group of 1,4-SNS is fully protonated; therefore, 1,4-SNS could not be effectively included in the CD cavity to exclude the cholic acid moiety. When the pH value was 6.2 or 7.2, its amino group and the imido group of the tether[24] in **1** are partially protonated. Therefore, there is the electrostatic repulsion interaction between the amino group of 1,4-SNS and the imido group of CD **1**'s tether, which makes the cholic acid moiety in **1** keep away from the CD cavity to give a good amphiphilic mode. This phenomenon is favorable for the formation of the nanoclusters. When the pH value was 10.0, the 1,4-SNS molecules could effectively exclude the cholic acid moiety from the CD cavity to form the nanoclusters.

4. Conclusions

In summary, we have prepared a class of porous nanospheres from amphiphilic cholic-acid-modified β-CD **1** using 1,4-SNS as guest molecules to trigger the assembly process. The 2D NMR and ICD studies as well as the K_S values indicate that a combination of the strong host−guest inclusion interaction and the shape of the guest molecule is essential for amphiphilic β-CD **1** to form the nanospheres. This work may not only open the way for the study of CDs in supramolecular self-assembled nanospheres but also may suggest a potential application of the nanosphere in drug/gas entrapment and release.

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Supporting Information Available: Nitrogen BET adsorption isotherm and TEM images of the porous nanospheres. This material is available free of charge via the Internet at http://pubs.acs.org.

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