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Self-Assembly Vesicles Made from a Cyclodextrin Supramolecular Complex

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Abstract: Self-assembly vesicles have been made from a cyclodextrin (CD) supramolecular complex, which is cooperatively formed with natural β-CD, 1-naphthylammonium chloride (NA), and sodium bis(2-ethyl-1-hexyl)sulfosuccinate (AOT) by weak noncovalent interactions. In the complex structure, a NA molecule is included inside a β-CD molecule while it is coupled with an AOT molecule on one side. The supramolecular structure and morphology of the vesicles were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS), respectively. The mechanism of vesicle formation and transition is discussed along with the data obtained from induced circular dichroism (ICD) and UV/visible spectroscopy, polarized optical microscopy (POM), and ^{1}H NMR spectroscopy. Both the fabrication and the transition of vesicles are controlled by the inclusion equilibria and the cooperative binding of noncovalent interactions, which include the "key–lock" principle, electrostatic interactions, π–π stacking, and amphiphilic hydrophobic association.

Keywords: cyclodextrins · noncovalent interactions · self-assembly · supramolecular · vesicles

Introduction

Supramolecular complexes formed through self-assembly are among the promising candidates for functional molecular devices and nanomaterials.[1] Extensive efforts have been directed toward self-assembly supramolecular complexes to explore their novel properties and functions which are not readily available without specific assembly of molecular components.[2] It is well known that various biological or chemical assemblies including vesicles, tubules, fibrils, and viral helical coats perform numerous biochemical actions in nature. Vesicles, in particular, have gained much interest for their applications as biomimetic models, drug or gene carriers, nanoreactors, etc.[3]

In supramolecular chemistry, cyclodextrin (CD) represents a type of host molecule consisting of a cyclic oligosaccharide with D-(+)-glucose as the repeating unit coupled by α-1,4-linkages. Common forms of CD include α-, β-, and γ-CDs with 6, 7, and 8 glucose units, respectively. It is known that the hydrophobic cavities of CDs can accommodate various types of micro- or macroguest molecules.[4] As a result of their unique structures and properties, CDs are closely related to many interesting topics, such as molecular recognition and encapsulation, self-assembly, and inclusions for different functional components.[5] In addition, CDs could be modified to form organized assemblies, such as monolayers, micelles, and vesicles.[6] Vesicles with combined properties of liposomes and macrocyclic host molecules offer great potential to encapsulate water-soluble molecules in the aqueous interior, to absorb hydrophobic molecules into the bilayer membrane, and finally to recognize and bind specific types of guest molecules through inclusions in CD cavities at the vesicle surface. However, such modification processes are, sometimes, complicated, nonreversible and time consuming. Therefore, it is desirable to develop facile methods for generating supramolecular functional materials from building blocks by noncovalent interactions,[7] such as van der Waals attractions, the "key–lock" principle, ionic interactions, amphiphilic association, etc.[8] The approach of noncovalent interactions is not only effective, but is also capable of making the assembling process reversible and is simpler compared to ones using covalently bonded systems. For example, reversible supramolecular transitions between the vesicles and nanotubes could be controlled by CD inclu-

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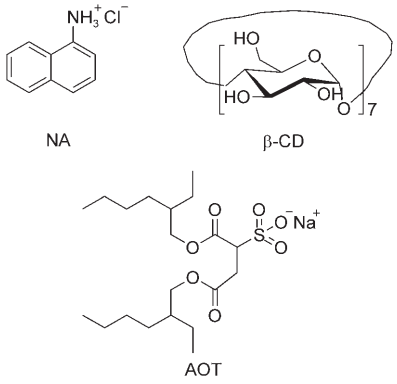
sions.[9] However, great challenges exist for assembling CD vesicles from natural CD and some other small segments by using noncovalent interactions.

In this work, a novel procedure is proposed to make self-assembled vesicles via a CD supramolecular complex, which is formed by including a 1-naphthylammonium chloride (NA) molecule inside a β-cyclodextrin (β-CD) cavity, which is then coupled with an anionic surfactant, aerosol AOT (AOT: sodium bis(2-ethyl-1-hexyl)sulfosuccinate) on one side. In the past decades, "catanionic" vesicles formed by



mixing cationic surfactants with anionic ones have been studied extensively,[10] and anionic AOT molecules were the subject of some of reports.[11] However, the formation of "catanionic" vesicles requires the use of surfactants with a hydrophobic chain of a sufficient length.[10a] Therefore, the use of NA molecules without long tails to counteract AOT ones could prevent the formation of "catanionic" vesicles. In this study, the organized processes of CD supramolecular complexes and vesicles are driven by several noncovalent interactions only. The obtained results may provide a better understanding of the design of such artificial supramolecular structures.

Results and Discussion

Different macroscopic phenomena: Vesicles were prepared by sonicating an aqueous solution of NA, β-CD, and AOT at room temperature. When the solution of AOT was slowly added into that of β-CD–NA inclusions, the mixture exhibited a slightly transparent opalescence. As a comparison, the direct mixing of NA and AOT aqueous solutions led to a cloudy suspension. As shown in Figure 1, the observed turbidnesses of vesicle solutions were much weaker than those of pure NA–AOT complex systems, indicating the fast formation of larger aggregates in nonvesicle systems.

Vesicles determined by TEM and DLS: The morphology and size of these self-assembled vesicles in water were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS), respectively. By using the

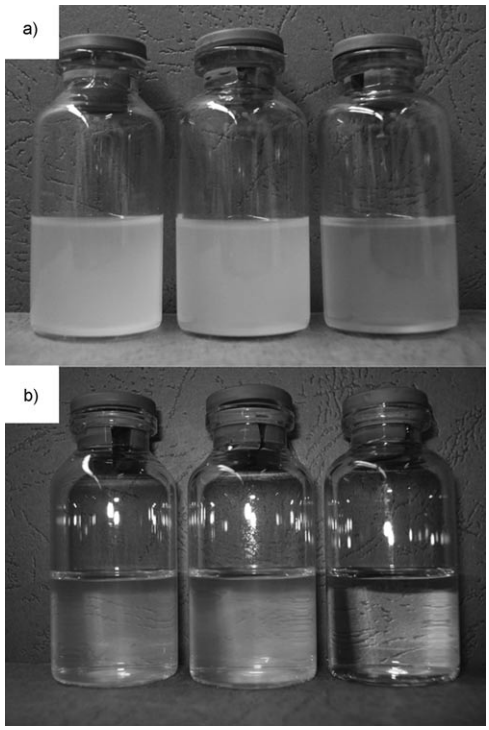


Figure 1. Photographs for systems of AOT/NA molar ratios = 1.0, 0.8, and 0.6, respectively (from left to right). a) Without β-CD and b) with β-CD.

uranyl acetate as a negative staining agent, we observed the closed spherical vesicles in all three systems as shown in Figure 2, with their outer diameters in the range of 30–200 nm. However, it was not possible to determine whether the vesicles were unilamellar or multilamellar. DLS measurements were also performed to measure the average diameters of vesicles and the results for three systems are listed in Table 1. Results obtained are consistent with the ones observed with TEM, showing an average hydrodynamic diameter of about 100 nm. Furthermore, the critical micellar concentration (cmc) of AOT at 25 °C was determined as 0.11 wt % by surface-tension measurements[12] and as 0.24 wt % by conductivity studies.[13] Between the cmc and concentration of 1.4 wt %, a single micellar phase can be formed.[14] For the systems in this study, the final AOT concentration was 1.0×10^{-3} mol dm^{-3} (that is, 0.044 wt %), far below its cmc. Therefore, it is believed that the vesicles could not be formed by the AOT itself. Consequently, a new kind supramolecular complex can be expected for the structure of observed vesicles. Besides the reactant concentrations used above, other systems with component concentrations of 1.0×10^{-4} or 1.0×10^{-5} mol dm^{-3} were also studied. But, there were not any obvious vesicular structures observed. Furthermore, β-CD would be observed as crystals under TEM if it was excessive.

ICD and UV/visible absorption spectra: An induced circular dichroism (ICD) spectrum was used to elucidate the guest molecule conformation, which is dominated by interactions

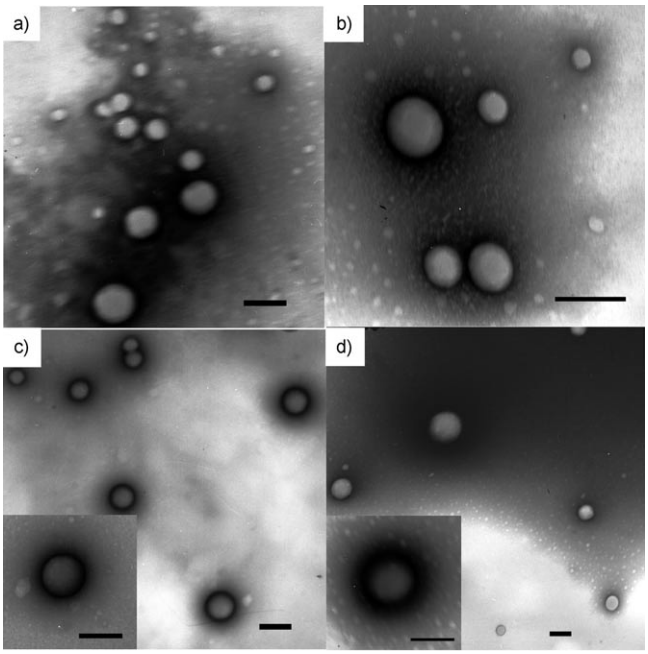


Figure 2. TEM micrographs of vesicles for the system with AOT/NA molar ratios = 1.0 (a) and (b), 0.8 (c), and 0.6 (d), respectively. Negative staining with UO_2Ac. Scale bars = 100 nm.

Table 1. Average hydrodynamic radius (R_h) of the vesicles determined by using light scattering.

c of AOT [$\times 10^{-3}$ mol dm^{-3}]	Molar ratio of AOT and NA	R_h [nm]
2.0	1:1	67.2
2.0	0.8:1	99.3
2.0	0.6:1	49.1

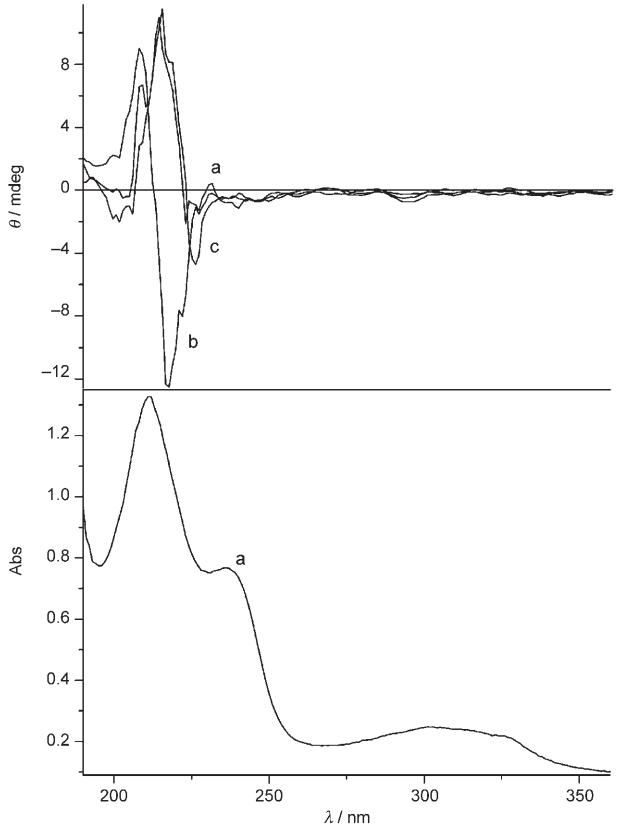


Figure 3. ICD (top) and UV/visible absorption (bottom) spectra of vesicle solutions for the system with AOT/NA molar ratios = 1.0 (a), 0.8 (b), and 0.6 (c), respectively. The UV/visible profiles of b and c are similar to that of a.

between the achiral chromophoric compound and the β-CD chiral cavity. As shown in Figure 3, all vesicle solutions display either positive or negative ICD signals, corresponding to the absorption maxima found in the UV/visible spectra (at approximately 212, 237, and 306 nm). However, neither the solution of NA or β-CD show any appreciable circular dichroism signal in the wavelength range of 190–400 nm. Therefore, it is considered that NA molecules are included in the cavities of β-CD, based on the general explanations of circular dichroism signals of CD complexes.[15] It is also possible that the CD inclusion process, by changing NA segments from hydrophobic to hydrophilic, plays an important role in the formation of vesicles. In addition, the ICD intensity shown in Figure 3b is much stronger than those of the others. This is partially consistent with the DLS data, in which R_h was measured as the maximum from the same solution. Based on these results, it can be concluded that more CD supramolecular complexes are assembled to vesicles at the molar ratio of AOT to NA = 0.8:1 among all three systems.

Equilibria and vesicle dissociation: Changes of the additives or environmental factors, such as temperature, concentration, and pH, will induce the transition of vesicles into other organized assemblies.[16] In our experiment, such vesicle transitions were found to be influenced by the stability of the CD supramolecular complexes. It should be noted that the formation process of inclusion complexes is actually a dynamic balance between the host CDs and guest molecules[17] with some of the guests being included or spread in the solution. Therefore, these simple complexes could be expected to form through a series of association equilibria. Corresponding equilibrium constants, denoted as K_n, are defined by Equations (1–5). The better space matching between the volumes of a NA molecule and the cavity of β-CD means that the latter is mainly occupied by NA,[18] and therefore, the equilibrium in Equation (5) could be ignored. In addition, at a much lower concentration, the NA–AOT complexes are easy to aggregate and separate out from the aqueous solution due to the stronger interactions between themselves. Therefore, the NA–AOT present in equilibrium shown by Equation (3) or (4) only represents a transition state, and will change to large aggregates rapidly. Under such circumstances, the equilibria in Equations (3) and (4) are unbalanced and both are inclined to form NA–AOT complexes, causing a gradual decrease of the NA and AOT concentrations and an increase in the CD concentration. From Equation (6), it can be easily recognized that such a concentration increase of CD cannot resist the effect of the

reduction of both NA and AOT concentrations. The subsequent decrease in the amount of CD–NA–AOT will gradually induce vesicle dissociation. Meanwhile, due to steric hindrance of the ammonium group at the 1-position of the naphthalene ring, NA molecules can only form a shallowly penetrated longitudinal inclusion complex or a weakly interacting lateral one,[19] which will also reduce the stability of inclusion complexes and accelerate the vesicle dissociation.

$$\mathrm{CD} + \mathrm{NA} \overset{K_1}{\rightleftharpoons} \mathrm{CD-NA} \quad K_1 = [\mathrm{CD-NA}]/([\mathrm{CD}][\mathrm{NA}]) \tag{1}$$

$$\mathrm{CD-NA} + \mathrm{AOT} \overset{K_2}{\rightleftharpoons} \mathrm{CD-NA-AOT}$$
$$K_2 = [\mathrm{CD-NA-AOT}]/([\mathrm{CD-NA}][\mathrm{AOT}]) \tag{2}$$

$$\mathrm{NA} + \mathrm{AOT} \overset{K_3}{\rightleftharpoons} \mathrm{NA-AOT}$$
$$K_3 = [\mathrm{NA-AOT}]/([\mathrm{NA}][\mathrm{AOT}]) \tag{3}$$

$$\mathrm{CD} + \mathrm{NA-AOT} \overset{K_4}{\rightleftharpoons} \mathrm{CD-NA-AOT}$$
$$K_4 = [\mathrm{CD-NA-AOT}]/([\mathrm{CD}][\mathrm{NA-AOT}]) \tag{4}$$

$$\mathrm{CD} + \mathrm{AOT} \overset{K_5}{\rightleftharpoons} \mathrm{CD-AOT}$$
$$K_5 = [\mathrm{CD-AOT}]/([\mathrm{CD}][\mathrm{AOT}]) \tag{5}$$

$$[\mathrm{CD-NA-AOT}] = K_1K_2[\mathrm{CD}][\mathrm{NA}][\mathrm{AOT}] \tag{6}$$

Crystal production from vesicle dissociation: In aqueous solutions, some NA molecules escape from the β-CD cavities, resulting in the dissociation of some vesicles. At the same time, the NA–AOT complexes are formed slowly through π–π stacking, electrostatic attractions, and hydrophobic effects.[20] Through the cooperative binding, the complex molecules are well oriented and produce several macroscopic flakes, which exhibit a high-order crystal structure as confirmed by the strong birefringence as shown in Figure 4b. These flakes appear colored and anisotropic under polarized light, indicating that, at the end of the transition, all the NA molecules are oriented in the same direction. Such oriented macroscopic flakes also demonstrate the perfect control of the molecular NA units. For comparison, when NA and AOT solutions were mixed directly, only white powder precipitates were observed due to the fast aggregation rate. Moreover, the ordered NA–AOT complexes produced due to the vesicle dissociation were analyzed by ^{1}H NMR spectroscopy as shown in Figure 5, in which the absence of β-CD signals in complexes supports the previous deduction that the supramolecular complexes of β-CD can dissociate. In addition, the stoichiometry between NA and AOT in the complexes is determined as a 1:1 molar ratio by comparing the peak integral intensities.

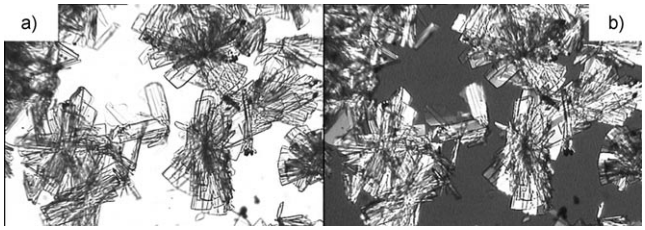


Figure 4. Micrographs of flakelike NA–AOT complexes under (a) normal, and (b) cross-polarized light.

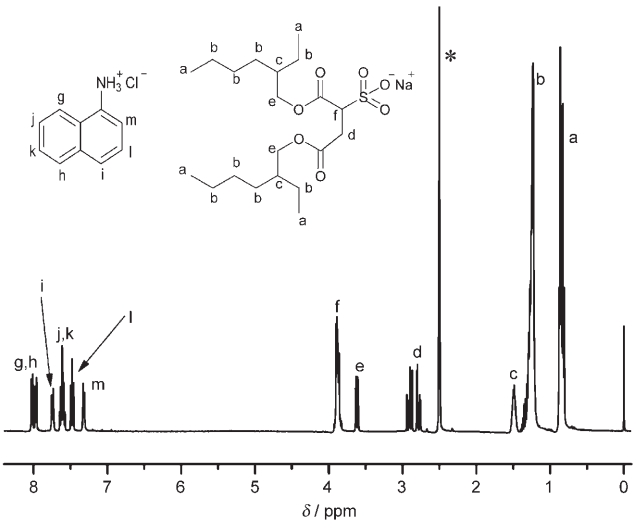


Figure 5. ^{1}H NMR spectrum of NA–AOT complexes in [D_6]DMSO. * denotes the solvent peak.

Formation and transition mechanism of vesicles: Based on the above experimental results, a possible self-assembly scheme is proposed as illustrated in Figure 6. The NA molecule is included by a β-CD and then attracts one AOT molecule by electrostatic interactions. Finally, they will form a CD supramolecular complex, which may be called the pseudoamphiphile. While being sonicated, the supramolecular complexes could self-assemble into vesicles. But some nonincluded or escaped NA molecules still exist and could attract AOT molecules to form complexes. This is a nonreversible process and will eventually break the equilibrium existing during the inclusion formation. In the meantime, some vesicles dissociate and some reassemble. Then, the newly formed NA–AOT complexes are organized through the noncovalent interactions mentioned above. They are oriented in the same direction, forming flakelike aggregates.

Conclusion

We have demonstrated a successful vesicle preparation from CD supramolecular complexes. Although a few reports have recently described vesicles composed entirely of nonionic, anionic, and cationic amphiphilic CDs, no chemical modification on CD is made in our scheme and a simple supramolecular self-assembly to produce the pseudoamphiphiles is utilized. Nonamphiphilic segments are driven to self-assemble via noncovalent interactions. NA molecules play the role of connectors, which couple β-CD (hydrophilic) and AOT (hydrophobic) by using inclusion effects and electrostatic interactions, respectively. Then the CD vesicles are formed under sonication. The properties of these vesicles

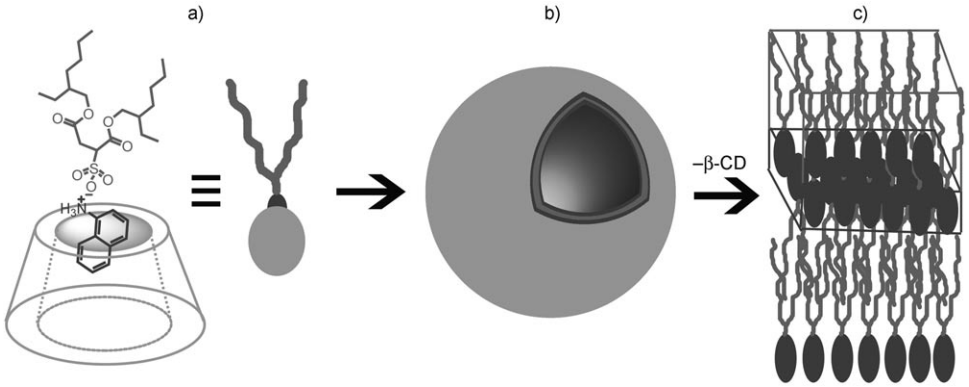


Figure 6. Schematic representation of vesicle formation from a CD supramolecular complex. a) Pseudoamphiphiles and b) vesicular self-assembly of pseudoamphiphiles. The gap is exaggerated to display the structure of a vesicular membrane. c) Aggregation of NA-AOT complexes.

are characterized by TEM, DLS, ICD, UV/visible, POM, and ^{1}H NMR spectroscopy, respectively. The mechanism analysis indicates that vesicle formation and transition are mainly controlled by the inclusion equilibrium. Although the stability of these vesicle systems is unsatisfactory, this interesting and valuable study and further improvement of the system should be helpful for the design of such supramolecular systems.

Experimental Section

Materials: β-CD (Sinopharm Chemicals), 1-naphthylammonium chloride (Merck), and sodium bis(2-ethyl-1-hexyl)sulfosuccinate (98%, Aldrich) were used as received. Water was triply distilled.

Techniques: Samples for TEM were prepared by a negative-staining technique with aqueous uranyl acetate solution. A Hitachi 100 CXII electron microscope was employed. DLS (dynamic light scattering) measurements were performed with a Wyatt Technology DAWN HELEOS instrument poised at 25°C by using a 12-angle replaced detector in a scintillation vial and a 50 mW solid-state laser ($\lambda = 658.0$ nm). All solutions were filtered through a 0.45 μm filter. Circular dichroism and UV/visible (ultraviolet-visible) spectra were recorded in a quartz cell (light path of 10 mm) on a JASCO J-810 spectropolarimeter and a HP 8453E instrument. ^{1}H NMR spectra were recorded on a Bruker AV-400 NMR spectrometer at room temperature. Chemical shifts of the complexes were referenced to $\delta = 2.50$ ppm for DMSO.

Vesicle preparation: The vesicles were prepared by sonicating the aqueous solution of NA, β-CD, and AOT at room temperature. First, the β-CD powder was added to the solution of NA (2.0×10^{-3} mol dm^{-3}, 5 mL) in an equimolar amount and the mixture was sonicated for 1 h. Then, different molar amounts of AOT were slowly dropped into the solution and the molar ratio of AOT to NA was 0.6, 0.8, and 1.0, respectively. After sonicating for another 1 h, the samples were kept at 25°C in an incubator.

Acknowledgements

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