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Enzyme-Responsive Molecular Assemblies Based on Host–Guest Chemistry

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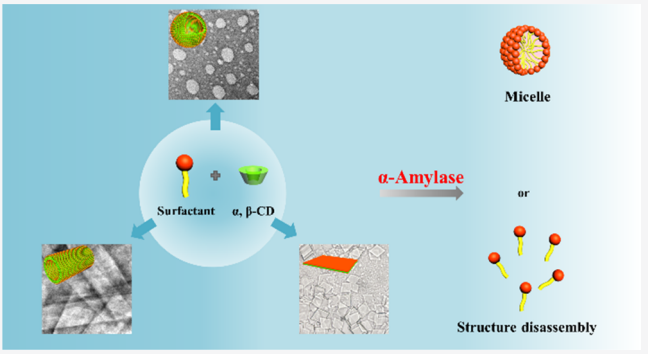
ABSTRACT: Recent years have witnessed a growing interest in the design of enzyme-responsive molecular assemblies that hold appealing applications in the fields of disease-related sensing, imaging, and drug delivery. Cyclodextrins (CDs) are amylase-cleavable host molecules that can associate with surfactants, alkanes, alkyl amines, fatty alcohols, and aromatic compounds to form diverse supramolecular structures. In this work, we report a versatile supramolecular platform to construct enzyme-responsive nanosystems via host–guest interactions, in which complexation between CDs and surfactants eventually leads to the formation of a variety of nanostructures such as vesicles and microtubes. These supramolecular structures are capable of loading water-soluble molecules or functional nanoparticles, which can be actively released on-demand in the presence of α-amylase. This universal strategy to fabricate enzyme-responsive supramolecular systems was further demonstrated with a range of surfactants with anionic, cationic, and nonionic headgroups. Our results highlight a versatile platform for the exploration of biologically responsive self-assembly with potential applications as controlled-release systems and microrobots.



INTRODUCTION

Enzyme-responsive molecular assemblies have attracted intensive attention in recent years.[1,2] Diversified responses to external stimuli can be achieved upon elegant molecular or supramolecular designs.[3] The dynamic nature of such systems is highly desirable in the fields of medicine, biotechnology, and materials science.[4] Currently, two groups of stimuli have been extensively studied for various applications of enzyme-responsive molecular assemblies: one is energetic stimuli such as temperature,[5,6] light,[7,8] and electromagnetic field[9,10] that do not change the system composition, and the other is material stimuli such as redox agents,[11,12] chemicals,[13,14] and enzymes[15−19] that introduce extra substances into the self-assembled systems. Compared to other stimuli, enzymes have advantages in terms of specificity and efficiency in response to the changes in physicochemical conditions. Besides, the abnormal expression of enzymes is often associated with human diseases. As a result, the exploration of enzyme-response assemblies is also of great significance in the fields of pharmacy and biotechnology.

In the past years, a number of enzyme-responsive molecular assemblies have been reported. For instance, Messersmith et al. designed a short-peptide substrate of transglutaminase (TGase) that can conjugate with a biocompatible polymer, leading to the formation of the polymer–peptide hydrogels within a few minutes.[20] Xu et al. constructed a series of enzymatic hydrogels using small amphiphilic molecules.[15,21] The development of enzymatic hydrogels has led to other structures, such as micelles[16] and vesicles.[22] To date, enzyme-triggered molecular assemblies have been widely utilized in folding and unfolding of peptides,[23,24] cell imaging,[25,26] and cancer therapy.[27,28] However, most existing efforts have been devoted to enzyme-triggered disintegration of molecular assemblies that are constructed either with peptides-modified polymers,[29,30] or with an enzyme substrate and other components.[31,32] In contrast, rarely studied are the enzyme-triggered structural transitions.[33,34] Moreover, most existing methods for the formation of enzyme-responsive assemblies require special synthesis, which is often costly and time-consuming. Besides, the complexity of chemical reactions hinders scientific understanding of the nature of the enzyme-responsive systems and further applications.

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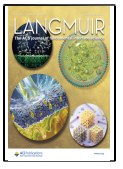

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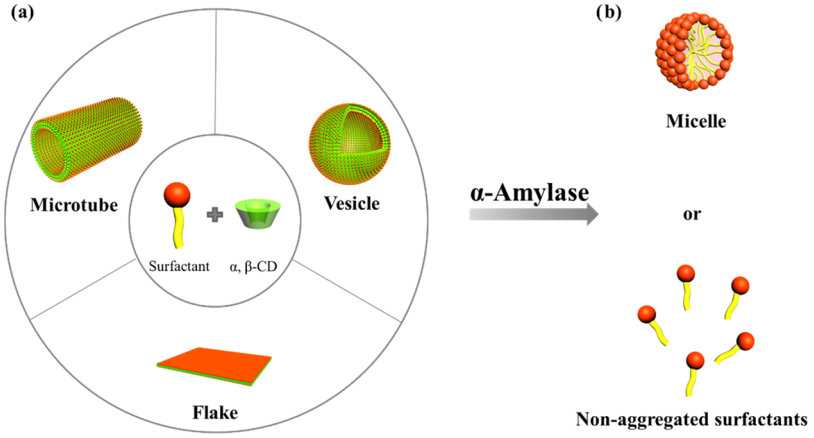
Scheme 1. Schematic Illustrations of Enzyme-Responsive Assemblies on the Basis of Surfactant−CD Complexes (a) and α-Amylase-Induced Degradation (b)



Herein, we construct a series of simple and effective structures based on the host−guest chemistry of cyclodextrins (CDs). CDs are macrocyclic compounds and considered biologically safe. Recent studies have shown that CDs are able to form host−guest complexes by associating with a vast number of commercially available materials.[35−44] In particular, we demonstrated previously that β-CD-amphiphile complexes can further assemble into diverse structures, including vesicles,[45−48] tubes,[45−48] lamellae,[45−48] helical ribbons,[49] and rhombics.[50] Because 1,4-glycosidic bonds in CDs can be cleaved by α-amylase,[51,52] we are able to construct the CD-based molecular assemblies that may serve as a versatile platform for the development enzyme-responsive systems, especially in the construction of enzyme-responsive assemblies that exhibit structural transitions.

Scheme 1 presents a general strategy to construct enzyme-responsive systems based on host−guest interactions. The structural transition can be achieved by simple mixing without any special synthesis. α-Amylase is able to transform many kinds of assemblies, e.g., vesicles, microtubes, and flakes. The enzyme-responsive systems can be formed from over a dozen systems, including conventional surfactants (anionic, cationic, and nonionic) with both α- and β-CDs. Moreover, the molecular assemblies vary in size ranging from nanometer (vesicles) to micrometer (microtubes and flakes) depending on the surfactant concentration. The wide size range is helpful to load the complexes with substances of different length scales (molecules and particles) and to achieve enzyme-triggered release.

EXPERIMENTAL SECTION

Materials. β-Cyclodextrin (β-CD, 96%), fluorescein isothiocyanate isomer−polystyrene (FITC−PS) microspheres (1.0 μm, 2.5% w/v), and sodium laurate (SL, 98%) were purchased from Aladdin. Sodium dodecyl benzene sulfonate (SDBS, 99%), α-amylase (from Aspergillus oryzae), dimethyl sulfoxide-d_6 (DMSO-d_6), and deuterium oxide (D_2O, 99%) were purchased from Sigma. Calcein, thioflavin T (ThT), sodium lauryl sulfonate ($SDSO_3$, 99%), dodecyl dimethyl benzyl ammonium chloride (DDBAC, 99%), *N*-dodecyl-β-D-maltoside (DDM, 96%), dodecyl trimethyl ammonium chloride (DTAC, 98%), Tween-20, and dodecyl pyridine chloride (DPyCl, 99%) were purchased from Macklin. Sodium dodecyl sulfate (SDS, 99%) was purchased from Acros. The dialysis bag (3500 D) was purchased from XYBo.

Sample Preparation. The samples were weighed according to the calculated amounts of SDBS, β-CD, and deionized (DI) water in the tubes. For the 8:16 mM vesicle system, the weights are 0.053 g of SDBS, 0.0347 g of β-CD, and 1.96 g of DI water, and for the 40:80 mM microtube system, the weights are 0.266 g of SDBS, 0.1734 g of β-CD, and 1.8 g of DI water. After vortexing for 30 s, the samples were heated at 70 °C until the solutions became transparent and isotropic; after that, the samples were incubated at 25 °C for at least 2 days before the following testing.

For the α-amylase reaction, the enzyme stock solution was prepared with a concentration of 8000 U/mL first. Then, 6.25 μL was added to 1 ml of 8:16 mM vesicle system and 1 mL of 40:80 mM microtube system, respectively; the enzyme concentration was 50 U/mL. At different time points, the solutions were heated at 100 °C to stop the enzymatic reaction and then cooled to room temperature before the following testing.

Fluorescence Spectrometry. Fluorescence spectra were recorded on a Hitachi F7000 spectrometer equipped with a constant temperature bath to control the temperature at 25 °C (λ_{ex} = 480 nm)

Nuclear Magnetic Resonance (NMR). The ^{1}H NMR experiments were performed on a Bruker ARX 500 MHz spectrometer with DMSO-d_6 as the solvent at 25 °C. By comparing the integration between β-CD (H1 protons, δ = 4.83 ppm) and the H proton on the benzene ring (δ = 7.12 ppm), the SDBS/β-CD complex ratio can be determined. The ROESY spectra experiments were performed on a Bruker ARX 600 MHz spectrometer at 25 °C with D_2O as the solvent.

Dynamic Light Scattering (DLS). DLS data were obtained by a NanoBrook NanoOmni instrument. The samples were filtered by 450 nm filters. All measurements were conducted using DI water at 25 °C.

Electrospray Ionization Mass Spectrometry (ESI-MS). ESI-MS measurements were carried out on an APEX IV FT-MS (Bruker). The operating condition of the ESI source was in the negative ion mode.

Atomic Force Microscopy (AFM). AFM measurements in the tapping mode under ambient conditions were conducted on a D3100 AFM (VEECO). One drop of the SDBS@2β-CD 40:80 mM solution was spin-coated on a mica surface and then placed at room temperature to dry before AFM observation.

Transmission Electron Microscopy (TEM). Samples were observed by a JEOL JEM 100CX, 80 kV, and a Tecnai T20, 200 kV. Drops of samples were put onto 200 mesh copper grids coated with a Formvar film. Excess water was removed by filter paper, and the samples were stained with 3% uranyl acetate for 3−5 min, and then, the excess stain was removed by a filter before TEM observation.

Confocal Laser Scanning Microscopy (CLSM). The samples were stained with thioflavin T (ThT) according to the following method. DI water, ThT, SDBS, and β-CD were weighed and transferred into the tubes, with the concentrations of ThT, SBDS, and β-CD being 1, 40, and 80 mM, respectively. The samples were consequently heated at 70 °C until they become transparent and isotropic. Then, the solutions were incubated at 25 °C for at least 48 h before testing. A drop of the samples was sealed between two slides, whose temperature was

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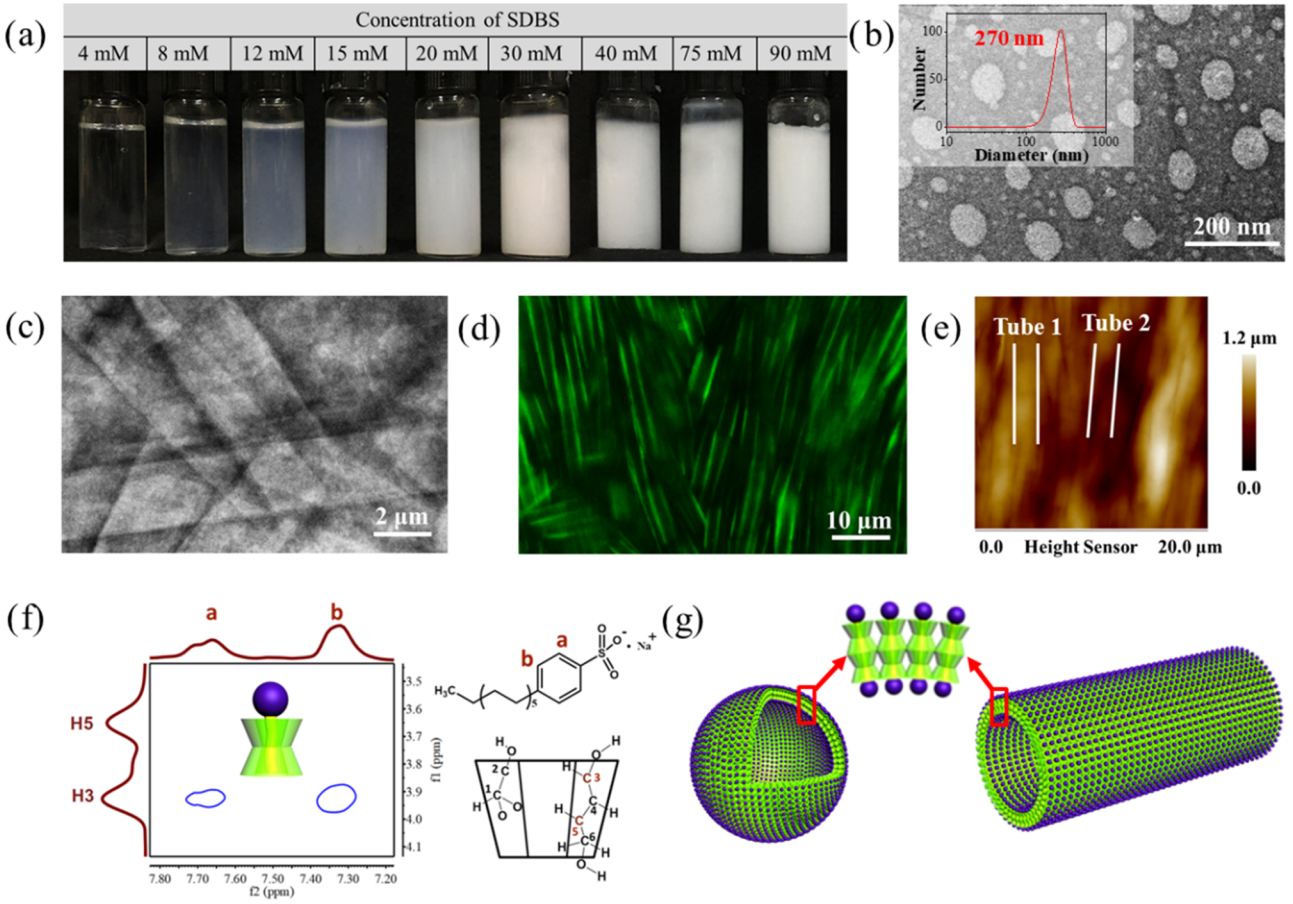

Figure 1. Host−guest complexation of SDBS and β-CD. (a) Photos of the host−guest systems at 25 °C; the molar ratio of SDBS and β-CD is 1:2. (b) TEM image and DLS result (inset) showing vesicle formation in SDBS@2β-CD (8:16 mM). (c) TEM image, (d) CLSM image, and (e) AFM image for the microtubes formed in SDBS@2β-CD (40:80 mM). (f) Two-dimensional (2D) NMR (Roesy, D_2O, 298 K) result of SDBS@2β-CD. (g) Schematic illustration showing the structural transformation of vesicles into microtubes in SDBS@2β-CD.

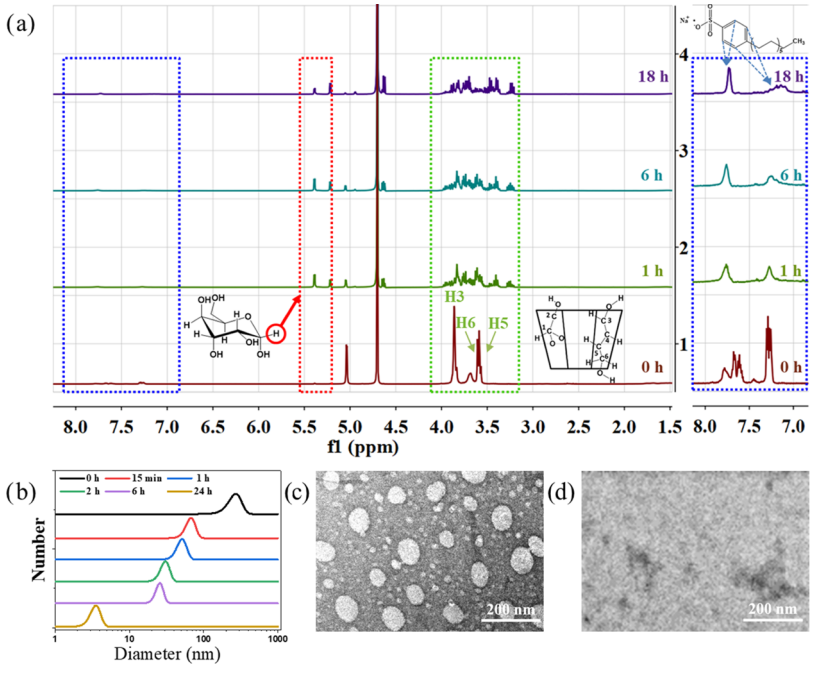

Figure 2. (a) NMR spectra showing the molecular structure changes during the α-amylase reaction. (b) DLS result showing the vesicles during the enzymatic reaction. TEM images of the vesicles (c) before and (d) after the enzymatic reaction for 24 h.

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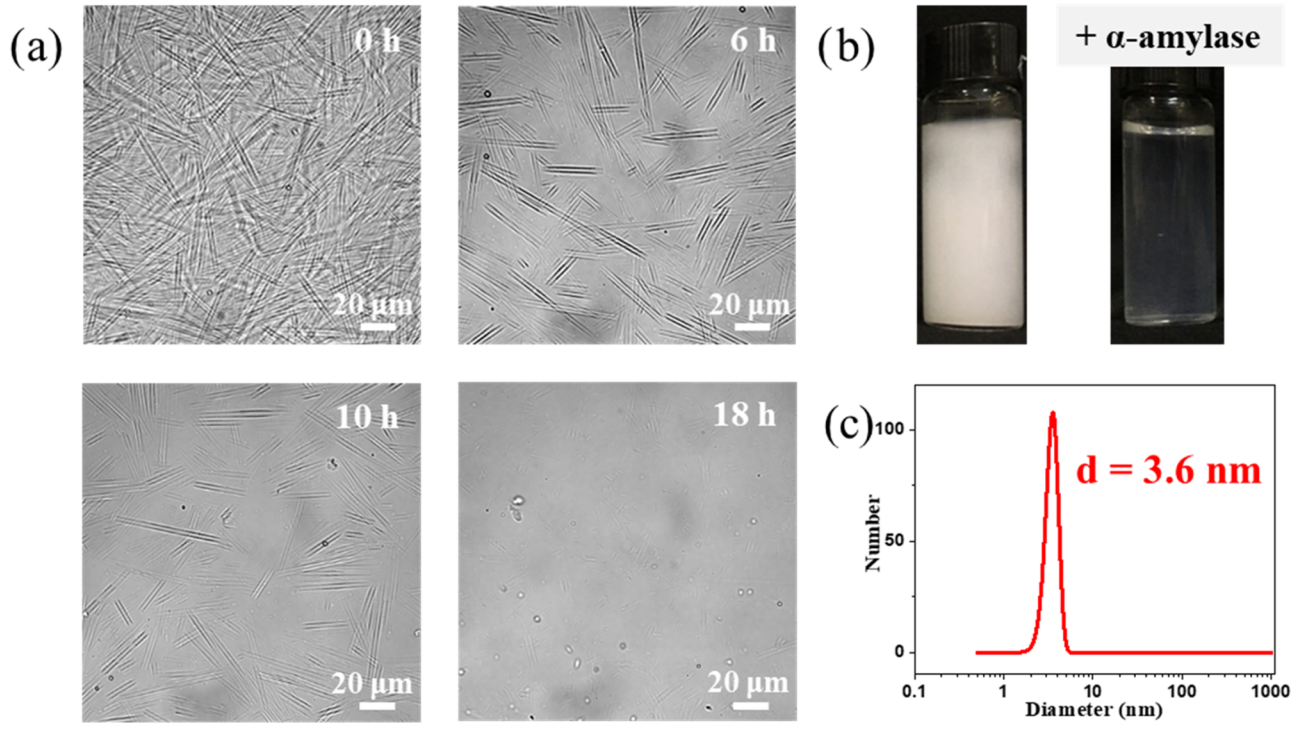

Figure 3. (a) Microscopy images showing α-amylase-triggered disassembly of microtubes in SDBS@2β-CD (40:80 mM). (b) Photos of the microtubes formed from the SDBS@2β-CD (40:80 mM) system before and after the enzymatic reaction. (c) DLS result of SDBS@2β-CD (40:80 mM) after the enzymatic reaction for 24 h.

controlled by a circulator bath. The CLSM experiments were conducted under fluorescence modes on A1R-si CLSM (Nikon, Japan).

Optical Microscopy. A drop of solution was sealed between two slides; then, images were captured by an LV100N polarizing microscope (Nikon Co.).

Dye Encapsulation. One milligram of calcein was mixed with 6 mL of SDBS@2β-CD 8:16 mM system at 70 °C for 20 min and then placed in a 25 °C incubator for at least 24 h. The solution was further transferred into the dialysis bag (MWCO = 3500) against water for 48 h to remove free calcein molecules.

Microsphere Encapsulation. One milliliter of 2.5% w/v FITC−PS microspheres was added to a 6 mL solution of SDBS@2β-CD (40:80 mM) at 70 °C to melt the microtubes and then sonicated to disperse the microspheres. The samples were cooled to room temperature for at least 48 h before use. Upon cooling, the samples were gently rotated to avoid the sedimentation of the microspheres.

RESULTS AND DISCUSSION

Construction of SDBS@2β-CD Assemblies. The host−guest inclusion complexes of sodium dodecyl benzene sulfonate (SDBS) @2β-CD were prepared by methods established in our earlier work.[45] Briefly, the desired amount of β-CD powder was added to an aqueous solution of SDBS at 70 °C at a stoichiometric ratio of 1:2. The system was then cooled to room temperature. Depending on the surfactant concentration, the resulting suspensions can be clear, opalescent, or whitish. The phase situation is illustrated in Figure 1a.

The TEM images reveal that vesicles were formed when the concentration of SDBS was in the range of 4−8 mM (Figure 1b), whereas tubes were observed when the concentration of SDBS was in the range of 30−90 mM (Figure 1c). Vesicles and tubes coexisted in between the two concentration ranges (Figure S1). Figure 1b shows the DLS results. It reveals that the average diameter of the vesicles formed in the 8 mM SDBS system is 270 nm, which is in line with the TEM observation. Figure 1c−e

Table 1. Assemblies of Anionic, Cationic, and Nonionic Surfactants with α- and β-CD and Their Enzyme Responses

	surfactants	CDs	assemblies	after α-amylase degradation
anionic	$SDSO_3$	α-CD	vesicle, microtube	micelle
		β-CD	vesicle, microtube	micelle
	SDS	α-CD	vesicle, microtube	micelle
		β-CD	vesicle, microtube	micelle
	SL	α-CD	vesicle, flake	micelle
		β-CD	vesicle, flake	micelle
cationic	DPyCl	α-CD	vesicle, microtube	micelle
		β-CD	vesicle, microtube	micelle
	DTAC	α-CD	vesicle, flake	micelle
		β-CD	vesicle, flake	micelle
	DDBAC	α-CD	vesicle, flake	micelle
		β-CD	vesicle, flake	micelle
nonionic	*N*-dodecyl-β-D-maltoside	α-CD	vesicle, flake	micelle
		β-CD	vesicle, flake	micelle
	Tween-20	α-CD	vesicle, flake	micelle or disassembly
		β-CD	vesicle, flake	micelle or disassembly

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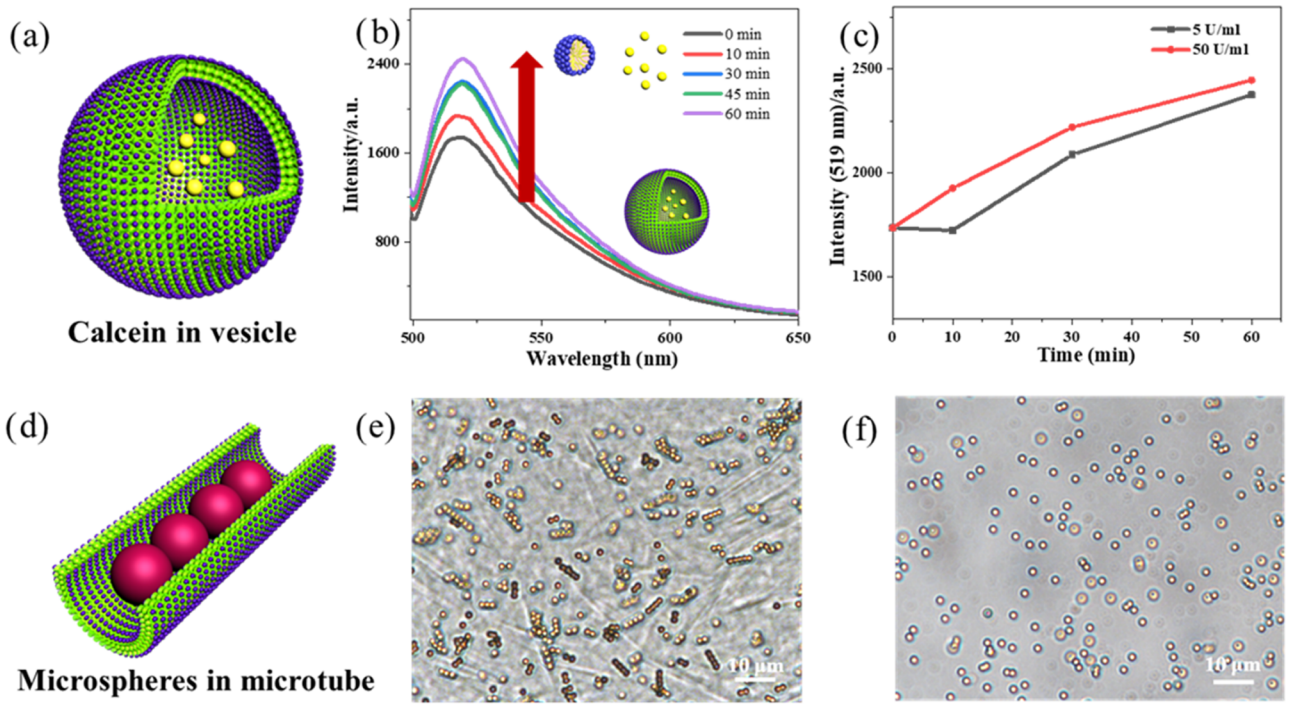

Figure 4. (a) Schematic illustration of water-soluble dye calcein encapsulated in SDBS@2β-CD vesicles. (b) Fluorescence spectra of the aqueous solution during the enzymatic reaction. (c) Variation of the fluorescence maxima with time at different concentrations of the enzyme. (d) Schematic illustration of the alignment of microspheres packed in the microtubes. (e) and (f) Microscopy images of microspheres in the microtubes before and after the 24 h enzymatic reaction.

shows the microscopic images of microtubes formed in the 40 mM SDBS system. These TEM images indicate that the average diameter of the microtubes is about 2 μm, which can be further confirmed by CLSM and AFM observations. Especially, CLSM clearly shows the hollow nature of the tubes and reveals that the length of the microtubes can be more than 30 μm.

^{1}H NMR measurements[53] (Figure S2) suggest that the binding ratio between SBDS and β-CD was 1:2 both in the 4:8 mM and 16:8 mM systems. The binding ratio was further confirmed by electrospray mass spectrometry (Figure S3). The peak position, corresponding to m/z = 2593.924065, is assigned to $[SDBS@2\beta\text{-CD}]^-$ (theoretical m/z = 2593.923817). As shown in Figure 1f, the two-dimensional NMR results indicate that the benzene ring of the SDBS molecule is closer to the H3 atom of β-CD, suggesting that the benzene ring is located on the wider rim of β-CD, as illustrated in the inset picture. The SDBS chain length is about two times the height of β-CD (Figure S4). The binding ratio between SDBS and β-CD is 1:2, namely, two β-CDs have threaded tail-to-tail onto the SDBS chain. Figures 1g and S5 illustrate the binding mode of SDBS and β-CD and the formation of vesicles and microtubes with the SDBS@2β-CD building blocks.

Enzyme-Responsive Transition from Vesicles and Microtubes to Micelles. Because β-CD can be hydrolyzed by α-amylase into glucose,[52] both SDBS@2β-CD vesicles and microtubes are enzyme-responsive. To demonstrate this, we added 50 U/mL α-amylase into the vesicle system and let it incubate at 25 °C. The hydrolysis of β-CD and the production of glucose were tracked by ^{1}H NMR.[54] Figure 2a shows that the peak positions of H3 (δ = 3.85 ppm), H5 (δ = 3.59 ppm), and H6 (δ = 3.6 ppm) from β-CD disappeared gradually, accompanied by the emergence of H (δ = 5.2 ppm) from glucose. MS analysis confirmed that the molecular structure of SDBS did not change during the enzymatic process (Figure S6). In line with this, DLS measurements revealed that the diameter of aggregates gradually reduced to 3.5 nm (Figure 2b). The final size was about the length of two SDBS molecules, indicating the formation of SDBS micelles. TEM images confirmed that no aggregate existed in the system after 24 h of hydrolysis (Figure 2c,d).

Similar transitions were observed in the system of microtubes. After addition of 50 U/mL α-amylase at 25 °C, the number of microtubes decreases gradually (Figure 3a). Meanwhile, the solution became transparent (Figure 3b). DLS measurement revealed the presence of micelles with a diameter of about 3.6 nm (Figure 3c). Control experiments suggested that the glucose generated by β-CD degradation is an assembly-inert substance (Table S1).

General Approach toward the Construction of Enzyme-Triggered Surfactant@CD Coassembly Systems. SDBS@2β-CD vesicles and microtubes display specific responsiveness to α-amylase. Figure S7 shows that both the vesicles and the microtubes remained intact within 24 h in the presence of other enzymes, such as butyrylcholinesterase (BchE). However, replacing SDBS with other amphiphiles would not affect the enzyme responsiveness. Table 1 shows the assemblies formed by other surfactants with CDs and their enzyme responses. In Figure S8, we show that anionic surfactants, such as sodium lauryl sulfonate ($SDSO_3$), cationic surfactants, like dodecyl pyridine chloride (DPyCl), and nonionic surfactant, such as *N*-dodecyl-β-D-maltoside (DDM), are all able to form supramolecular vesicles and microtubes (or flakes) with α- or β-CD and that all these structures display specific α-amylase-responsiveness.

Enzyme-Triggered Cargo Release from Vesicles and Microtubes. Vesicles are well-known carriers for molecular

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drugs. To demonstrate the carrying capability of vesicles assembled from host−guest supramolecules, we added calcein, a water-soluble fluorescent dye, to an aqueous solution of SDBS@2β-CD at 70 °C. The mixture was then cooled at room temperature (Figure 4a). TEM and DLS results suggested that calcein encapsulation did not affect the size and morphology of the vesicles (Figure S9). Upon the addition of α-amylase, the fluorescence intensity of the aqueous solution was enhanced gradually (Figure 4b). The higher the enzyme concentration, the stronger the fluorescence intensity observed at the same reaction time (Figure 4c), indicating that the release of calcein is closely affiliated with the enzymatic hydrolysis of the vesicles. The stability of the encapsulation in the presence of 20 U/mL cholinesterase was verified by fluorescence spectra; the intensity of the solutions showed no change during 24 h (Figure S10).

We observed similar enzyme-responsive behavior for the microtubes. Because the diameter of microtubes is around 2 μm, they could accommodate microspheres with diameters of up to 1 μm, fluorescein isothiocyanate isomer−polystyrene (FITC−PS) microspheres were packed into the microtubes with the same method as reported in the previous work.[55] Figure 4d shows that the microspheres align in a single file in the microtubes. After enzymatic hydrolysis, the microtubes disappeared, releasing the microspheres into the solution (Figure 4e,f). The directional arrangement and transportation of microspheres are useful in creating functional nanorobots.

CONCLUSIONS

We have developed a general approach to construct enzyme-responsive systems using different kinds of surfactants (cationic, anionic, nonionic) and CDs (α and β). Diverse structures can be assembled by simple mixing and then disassembled by leveraging the specific α-amylase-responsiveness of CDs. The molecular assemblies can be transformed into micelles or totally disassembled due to the disappearance of the host−guest interactions. In fact, we anticipate that double or triple enzyme responsiveness can be achieved when the enzyme substrates act as the guest molecules. Furthermore, the assembled structures can be loaded with molecules and nanoparticles due to the variety and multiple length scales (from nanometer to micrometer) to achieve the enzyme-triggered release. This general strategy provides a powerful platform not only for the construction and application of molecular assemblies but also for opening new vistas to enzymatically controlled release of drugs and functional particles including nanorobots.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.langmuir.1c01226.

TEM, optical microscopy, and AFM images; ^{1}H NMR spectra; fluorescence spectra; ESI-MS results; and DLS results (PDF)

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Author Contributions

The manuscript was written through the contribution of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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