Supramolecular Chemistry

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Polymeric Core–Shell Assemblies Mediated by Host–Guest Interactions: Versatile Nanocarriers for Drug Delivery**

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Polymeric assemblies, such as micelles, vesicles, nanofibers, and macroscopic tubes, have attracted great attention in recent years.[1] These organized assemblies, which have sizes that range from the nanoscale through the microscale to the macroscale, have found wide applications in areas such as bioengineering, biomedicine, materials science, and pharmaceutics.[2] Among these diverse assemblies, polymeric micelles and micelle-like core–shell nanospheres are recognized as very promising nanocarriers for drug and gene delivery.[3] The efficacy of several such nanocarriers for antitumor drugs has been demonstrated by intensive studies in preclinical and clinical trials.[4] In general, polymeric core–shell nanospheres are assembled in an aqueous solution by utilizing the hydrophobic interactions between core-forming segments.[5] The hydrophobic inner core serves as a nanocontainer for hydrophobic drugs, while the outer shell, which comprises hydrophilic polymers such as polyethylene glycol, provides the colloidal stability. Pioneering work by Harada and Kataoka,[6a] and Kabanov and co-workers,[6b] showed that the interactions between core forming segments of block copolymers can be electrostatic. In addition, metal–ligand coordination and hydrogen-bonding interactions can also drive the formation of nanoassemblies.[7] Herein we report the successful engineering of novel core–shell nanospheres directed by inclusion interaction between a host macromolecule and a guest substance. β-Cyclodextrin (β-CD) was selected as a host unit for the construction of a hydrophilic host block that is covalently linked with another hydrophilic segment, while hydrophobic substances, either small molecules or macromolecules, are employed as guest components. Core–shell nanoassemblies can form by host–guest interaction mediated spontaneous assembly in an aqueous solution (Figure 1). These novel assemblies might be used as versatile nanocarriers, considering the excellent inclusion-solubilization performance of β-CD to many hydrophobic drugs.[8] In addition, this strategy circumvents the relatively complicated synthetic procedure frequently required for conventional polymeric micelles in order to achieve the desirable encapsulation for a specific drug, since the loading capability is mainly determined by the compatibility between a drug and the hydrophobic segment.[9]

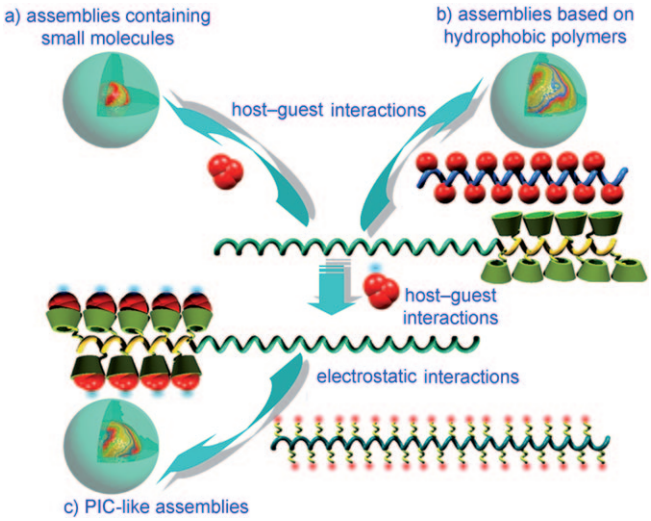

Figure 1. Schematic illustration of the formation of various host–guest assemblies: a) small hydrophobic molecules mediated assemblies, b) assemblies formed in the presence of a hydrophobic polymer, and c) PIC-like assemblies.

As a proof of concept, we synthesized a diblock hydrophilic copolymer characterized by tandem alignment of a polyethylene glycol (PEG) block and a polyaspartamide block carrying β-CD units on the side chain (PEG-*b*-PCD; see Figure S1a in the Supporting Information). α-Methoxy-ω-amino-PEG ($M_w = 5000$) was selected as a hydrophilic segment. A block copolymer (**1**) with a polyaspartamide block that contained ethylenediamine (EDA) units (PEG-*b*-PEDA) was synthesized following previously reported procedures.[10] The ^{1}H NMR spectrum of **1** indicates that the PEDA block consists of about 12 structural units, which is consistent with the result from MALDI-TOF mass spectrometry (see Figure S2b in the Supporting Information). β-CD was then covalently linked to PEG-*b*-PEDA to obtain PEG-*b*-PCD (**2**). The reaction was followed by using ^{1}H NMR spectroscopy, which indicated that the temperature and reaction time were important factors in the conjugation of β-CDs onto the PEDA segments. Optimized reaction conditions, that is, use of an excess of 6-monotosyl β-CD at 70 °C for 5 days, were

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adopted to synthesize **2**. Under these conditions, a highly efficient conjugation (up to 90%) can be achieved; this is especially true for copolymers with relatively short PEDA chains. Calculations based on the ^{1}H NMR spectrum of **2** suggest that about 10 β-CD molecules were introduced into the side chains of the PEDA segment (^{1}H and ^{13}C NMR spectra are shown in Figure S1b in the Supporting Information), which agrees to a certain extent with the MALDI-TOF mass spectrometry results (Figure S2c in the Supporting Information). The resulting polymer **2** can be easily dissolved in water at room temperature. It can also be easily dissolved in DMSO when heated to about 50 °C, which was evidenced by temperature-dependent ^{1}H NMR spectra recorded in [D$_6$]DMSO (Figure S3 in the Supporting Information).

The formation of polymeric assemblies mediated by the host–guest interactions between PEG-*b*-PCD and hydrophobic substances was initially investigated by using pyrene as a guest molecule. The normalized emission spectra of pyrene in aqueous solutions that contained PEG-*b*-PCD are shown in Figure 2a. As the concentration of PEG-*b*-PCD was

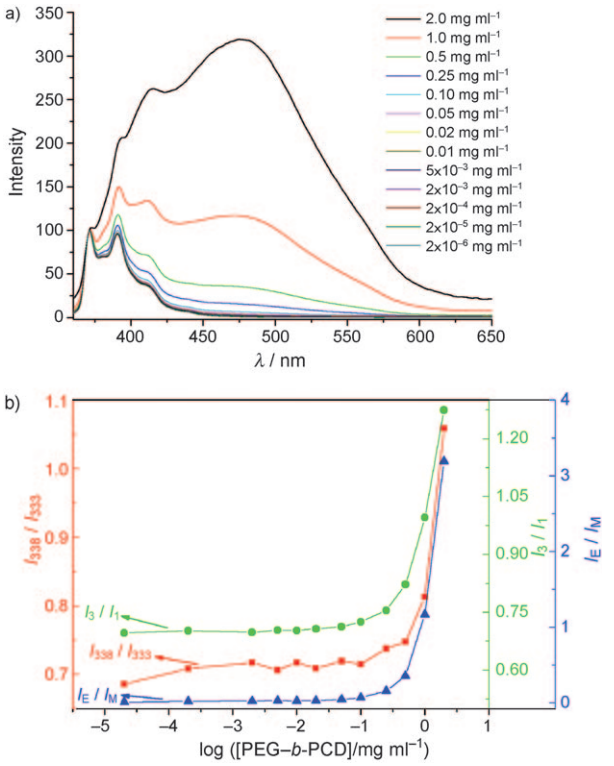

Figure 2. Normalized emission spectra of pyrene in aqueous solutions containing PEG-*b*-PCD. a) Emission spectra with an excitation wavelength at 339 nm and b) plots of I_{338}/I_{333}, I_3/I_1, and I_E/I_M as a function of PEG-*b*-PCD concentration. [Pyrene] = 6.0×10^{-7} M.

increased, a significant enhancement in excimer intensity (420–600 nm) could be observed. In the case of β-CD, however, no excimer formation can be observed (Figure S4 in the Supporting Information). It is well known that excimer formation is a short-range phenomenon (3–5 Å); pyrene excimers are formed either by the collision between excited- and ground-state monomers or by the excitation of preassociated pyrene pairs in the ground state.[11] For PEG-*b*-PCD, the excimer formation is likely to arise from the latter process. The broadening of the excitation band of pyrene in the presence of PEG-*b*-PCD supports the ground-state dimer formation (Figure S5 in the Supporting Information).[12] Additionally, the significant decrease in the excitation intensity of PEG-*b*-PCD solution is likely to result from the quenching phenomenon, which suggests the existence of a high local concentration of pyrene. The excitation spectrum of pyrene monitored at 475 nm shows a significant bathochromic shift with broadening in the vibrational structure compared with the excitation spectrum monitored at 390 nm, which also supports the ground-state dimer formation of pyrene (Figure S6 in the Supporting Information).[12] Plots of concentration-dependent changes in intensity ratios I_{338}/I_{333} (the intensity ratio of the (0,0) band in pyrene excitation spectrum), I_3/I_1 (the intensity ratio between the third and first vibrational bands in pyrene emission spectrum), and I_E/I_M (the intensity ratio of the excimer signal (475 nm) to the monomer signal (371 nm) in the emission spectrum) are shown in Figure 2b. Significant increases in the values of I_{338}/I_{333}, I_3/I_1, and I_E/I_M can be observed for pyrene as the concentration of PEG-*b*-PCD increased to a certain point (Figure 2b). No significant changes in I_E/I_M, however, were found in the case of β-CD (Figure S4b in the Supporting Information). Furthermore, the values of I_3/I_1 for PEG-*b*-PCD are significantly larger than those for β-CD. These observations indicate that pyrene molecules are located in a more hydrophobic microenvironment in PEG-*b*-PCD.[13] The apolar cavity of β-CD should be responsible for the changes in fluorescence spectra of pyrene in aqueous β-CD solutions. In the case of PEG-*b*-PCD, in addition to the apolar cavity of β-CD, the association of pyrene molecules should also contribute to the enhanced local hydrophobicity as evidenced by excimer formation. These results suggest that PEG-*b*-PCD may have formed core–shell assemblies in the presence of hydrophobic pyrene.

To further elucidate the characteristics of this type of assemblies, PEG-*b*-PCD assemblies that contained pyrene (0.6 wt%) were prepared by using a dialysis procedure (height and 3D AFM images of the pyrene-containing PEG-*b*-PCD assemblies are shown in Figure S7 in the Supporting Information). These AFM images show assemblies of a round shape with diameters in the range 20–120 nm. Atomic force microscopy (AFM) sectional analysis (Figure S8a in the Supporting Information) showed that the diameters of the assemblies were generally ten to seventeen times larger than the heights of the aggregates. This result should be attributed to the flattening of spherical particles upon adsorption onto the mica surface and indicates that these assemblies are soft enough to deform upon drying;[15] furthermore, the tip convolution effect could also be responsible for this phenomenon.[16] Calculations based on AFM images gave a mean aggregate size of 63.5 nm, while dynamic light scattering (DLS) measurements showed a mean diameter of 27.3 nm (Figure S9a in the Supporting Information). In addition, transmission electron microscopy images reveal the mean assembly size to be about 20.0 nm (Figure 3a), which is in good agreement with the DLS result. A similar phenomenon

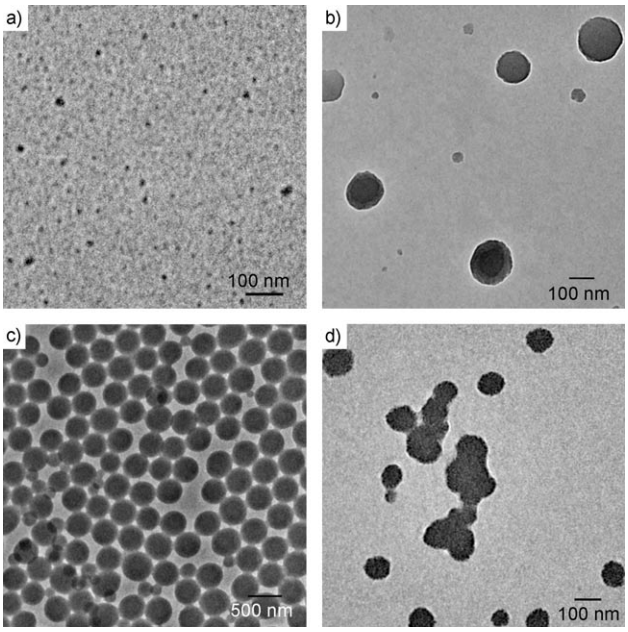

Figure 3. TEM images of assemblies based on PEG-*b*-PCD containing a) Pyrene, b) PBLA, (PBLA/PEG-*b*-PCD 1:20), (c) PBLA (PBLA/PEG-*b*-PCD 8:20), and (d) ADCA and PEI.

of simultaneous encapsulation and assembly formation was also observed for PEG-*b*-PCD in the presence of other hydrophobic compounds such as indomethacin (IND, a nonsteroidal anti-inflammatory drug) and coumarin 102 (Figures S8b, S9b, and S10 in the Supporting Information). A preliminary in vitro release study was performed to demonstrate the chemically stimulated release behavior of assemblies based on PEG-*b*-PCD and IND. As shown in Figure 4, in

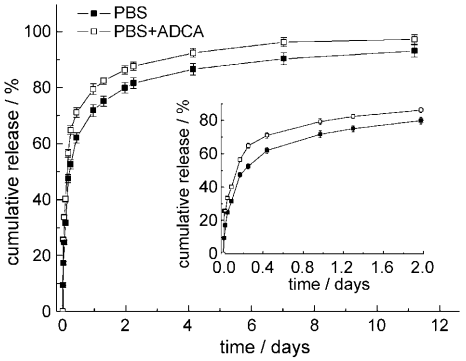

Figure 4. In vitro release profiles of assemblies based on PEG-*b*-PCD and IND (12.8 wt%). Release experiments were performed in phosphate buffered saline (PBS; 0.1 M, pH 7.4) and PBS containing ADCA (0.05 M) respectively.

the presence of adamantane carboxylic acid (ADCA), a competitive guest molecule with a higher complexation constant than that of IND, the drug release rate was accelerated. This effect was gradually leveled off in the later release stage.

As an initial conclusion, the above results suggest that core–shell aggregates could be assembled by PEG-*b*-PCD in an aqueous solution in the presence of a hydrophobic compound, a process that is mediated by host–guest interactions between the cyclodextrin and the hydrophobic compound. After the insertion of a hydrophobic group into the apolar cavity, the protruding portion provides the hydrophilic–hydrophilic block copolymer with localized hydrophobicity, which leads to the formation of a pseudo-amphiphilic block copolymer; that is, the hydrophobic compounds hydrophobilize the PCD blocks of the PEG-*b*-PCD copolymers. Further assembly of this pseudo-amphiphilic copolymer in aqueous solution may form the core–shell nanoassemblies. Additional free hydrophobic molecules can be simultaneously encapsulated in the cores of assemblies by the hydrophobic interaction. This process is schematically illustrated in Figure 1 a.

We next focused on the assembly behavior of PEG-*b*-PCD in the presence of a hydrophobic polymer. A previous report by Wang and Jiang shows the formation of micelle-like aggregates by a β-CD-containing homopolymer and a polymer with an adamantyl side group.[17] We therefore selected poly(β-benzyl L-aspartate) (PBLA) with M_n of 2000 as the model guest polymer (Figure S2d in the Supporting Information). Assemblies based on PEG-*b*-PCD/PBLA were prepared by using a dialysis procedure, with DMSO as a common organic solvent. As shown in Figure 3 b, spherical assemblies with mean diameters that ranged from 50 to 200 nm were obtained with a theoretical feed ratio of 1:20 (weight ratio of PBLA to PEG-*b*-PCD); analysis based on TEM images indicated the mean assembly size to be about 96.4 nm. This value agrees with that determined by DLS (118.7 nm; Figure S9c in the Supporting Information). On the other hand, SEM studies indicated the mean assembly size to be about 256.7 nm (Figure S11 in the Supporting Information). This disagreement should also be due to the flattening of assemblies when they were dried on the mica surface. For assemblies prepared with a feed ratio of 8:20, the particle size increased significantly, as observed from TEM image shown in Figure 3 c. The mean size was determined by DLS to be 209.2 nm (Figure S9d). The cores of these assemblies were investigated by ^{1}H NMR spectroscopy and fluorescence anisotropy measurements; no signals corresponding to PBLA can be observed for PEG-*b*-PCD/PBLA assemblies in D_2O (Figure S12 in the Supporting Information). However, signals at 7.3 and 5.0 ppm, which are characteristic peaks of protons of the benzyl group, are evident when a DMSO solution is used. This indicates that the cores of these assemblies are mainly composed of PBLA. In addition, staining using phosphotungstic acid (PTA) enabled direct observation of the core–shell structure of these assemblies. As shown in Figure 5 b, a shell can be clearly observed, and the shell thickness is almost a constant for all assemblies regardless of their particle size. Statistical analysis of the TEM images shows the average thickness of shells to be about 30 nm. This result suggests that the shells of the assemblies are mainly composed of substantially extended PEG chains since PTA preferentially stains the hydrophilic domains and the measured shell thickness is similar to the length of the PEG

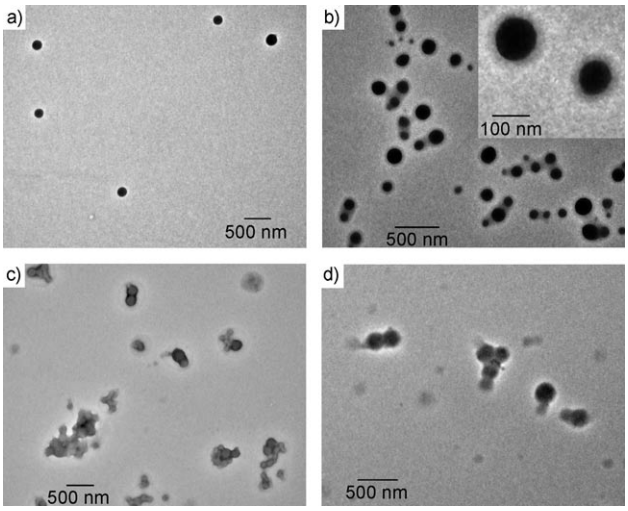

Figure 5. TEM images of assemblies based on a PEG-*b*-PCD/PBLA ratio of 15:6 a) control, b) staining with PTA, c) in the presence of potassium iodide (0.05 M), and d) in the presence of benzyl alcohol (0.05 M).

blocks. Further information on the microviscosity of the inner core was provided by the depolarization of fluorescence using 1,6-diphenyl-1,3,5-hexatriene (DPH) as a fluorophore. The anisotropy value (r) measured for DPH in aqueous solution of PEG-*b*-PCD/PBLA assemblies is 0.2, while the r value is 0.25 for PEG-*b*-PBLA micelles, which suggests that the former exhibits almost the same microviscosity as that of the latter. These results demonstrate that the cores of assemblies based on PEG-*b*-PCD and PBLA are essentially rigid. The rigid cores provide these assemblies with dynamic stability against dilution,[9a] which is very important for drug delivery systems based on polymeric assemblies to be administered by systemic injection. In addition, lyophilized samples of PEG-*b*-CD/PBLA assemblies can be redispersed in water without significant increase in the mean size. Through the same procedure, assemblies can also be prepared using poly(D,L-lactide) (PDLLA) as a guest polymer (Figures S9e and S13 in the Supporting Information). Additionally, by using PEG-*b*-PCD/PBLA-based assemblies as nanocarriers, sustained in vitro release of coumarin 102 was achieved (Figure S14 in the Supporting Information).

Considering the reversibility of host–guest interaction between β-CDs and the guest molecules, we investigated the effect of small molecule stimulants on the PEG-*b*-PCD/PBLA assemblies. Two types of stimulants, potassium iodide and benzyl alcohol (BA), were selected because both of them can form inclusion complexes with β-CD.[18] As illustrated in Figure 5c,d, interparticle aggregates were formed in the presence of either potassium iodide or BA, with morphologies that were significantly different from those of the original separate assemblies (Figure 5a). The chemical-triggered aggregation of the assemblies is likely to arise from the deshelling effect that results from the competition between small stimulants and PBLA to complex with β-CD groups. A similar aggregation effect was observed when these assemblies were exposed to CTAB, a surfactant that can also interact with β-CD (Figure S15 in the Supporting Information). This unique characteristic might be beneficial in drug delivery, considering the PEG dilemma in drug/gene delivery, that is, the PEG shell can stabilize nanoparticles in circulation, but it can also impede the intracellular trafficking.[19] The new stimulant-sensitive assemblies may both improve the stability in circulation and facilitate the subsequent intracellular trafficking upon partial dePEGylation.

Based on these results, we can conclude that well-defined core-shell assemblies can be successfully prepared by PEG-*b*-PCD copolymers and polymers with appropriate hydrophobic groups. The mechanism for the formation of assemblies based on PEG-*b*-PCD and a hydrophobic polymer is illustrated in Figure 1b. As the dialysis proceeds, the common solvent (DMSO in this case) diffuses out and water diffuses into the polymer-rich phase. The presence of PEG-*b*-PCD will decrease the surface tension between the guest polymer-based nanoparticles and outer water phase, which prevents the otherwise large-scale aggregation of the hydrophobic molecules. As a result, free guest macromolecules and the guest macromolecules associated with β-CD containing blocks form the cores of resultant nanoparticles, while PEG chains act as a hydrophilic shell to stabilize the assemblies.

Polyion complex (PIC) micelles, as reported by Kataoka and co-workers,[2c,20] and Kabanov and co-workers[6] have attracted great attention because of their potential applications in biomedicine and pharmaceutics. Accordingly, it is interesting to construct PIC assemblies by the host–guest interactions of PEG-*b*-PCD with guest molecules. For a preliminary study, ADCA was selected as a guest molecule, since the strong inclusion interaction of the adamantyl group with β-CD has been well demonstrated.[21] A pseudo-polyelectrolyte copolymer with one negatively charged block was initially prepared by taking advantage of the host–guest interaction between PEG-*b*-PCD and ADCA, further electrostatic interaction of this supramolecular polyelectrolyte and polyethylenimine (PEI) led to the formation of PIC-like assemblies with polyelectrolyte complex cores that comprised PEI and an ADCA-complexed block of PEG-*b*-PCD (Figure 1c). TEM observations indicated that these assemblies were spherical with a mean diameter of 100.7 nm (Figure 3d); the mean size was determined to be 97.1 nm (Figure S9f in the Supporting Information) by DLS. These results demonstrate that PEG-*b*-PCD copolymers may be used to construct potential delivery vectors for water-soluble macromolecules such as proteins, therapeutic DNA, and siRNA.

In conclusion, a novel hydrophilic–hydrophilic block copolymer has been synthesized. The utilization of such a novel diblock copolymer with a PEG block and a block bearing β-CD side groups has been demonstrated to assemble into novel and versatile core–shell nanocarriers. The β-CD conjugated block serves as the host segment that can form inclusion complexes with hydrophobic substances, while the hydrophilic segment can impart the resultant assemblies with stability. Host–guest recognition mediated nanoassemblies with hydrophobic inner cores and hydrophilic palisades can be prepared by a β-CD containing copolymer and hydrophobic small molecules or hydrophobic polymers. Furthermore, with the proper selection of charged guest molecules,

PIC-like assemblies can also be constructed by using this type of copolymers. Based on the well-established solubilization effect of various cyclodextrins (α, β, or γ) to a broad range of hydrophobic compounds, cyclodextrins bearing block- or graftlike copolymers with one stabilizing/hydrophilic segment may be developed into new types of universal nanocarriers. Additionally, assemblies based on this procedure exhibit chemical sensitivity, which might be useful for responsive delivery and bio- or chemical sensing.

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