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Host-Guest Interaction Mediated Polymeric Core-Shell Assemblies: Versatile Nanocarriers for Drug Delivery

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Abstract

Novel hydrophilic-hydrophilic block copolymer with one block containing β -cyclodextrin was synthesized. Core-shell structured nano-assemblies with chemical sensitivity could be constructed by this copolymer in the presence of hydrophobic compounds. By selecting appropriate guest components, polyion complex micelles could also be assembled. These results suggest the potential versatile applications of this type of copolymers in pharmaceuticals, nanomedicine, and nano-biotechnology.

Keywords

Host-guest interaction; assemblies; nanocarriers; drug delivery; chemical sensitivity

Polymeric assemblies, such as micelles, vesicles, nanofibers and macroscopic tubes, have attracted great attention in recent years.[1] These organized assemblies ranging from nanoscale, micro- to macroscale have found wide applications in areas such as bioengineering, biomedicine, materials science and pharmaceuticals.[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] Several such nanocarriers for antitumor drugs have been intensively studied in preclinical and clinical trials, and their efficacy has been demonstrated.[4] Generally, polymeric core-shell nanospheres are assembled in an aqueous solution utilizing the hydrophobic interactions between coreforming segments.[5] The hydrophobic inner core serves as a nanocontainer for hydrophobic drugs, while the outer shell comprised of hydrophilic polymers, such as polyethylene glycol, provides the colloidal stability. Pioneer works by the groups of Kataoka and Kabanov showed that the interactions between core forming segments of block copolymers can be electrostatic.[6] In addition, metal-ligand coordination and hydrogen-bonding interactions can also drive the formation of nano-assemblies.[7] In this study, novel core-shell nanospheres directed by inclusion interaction between a host macromolecule and a guest substance, are successfully engineered. β -Cyclodextrin (β -CD) is selected as a host unit to build one 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 nano-assemblies can form by host-guest interaction mediated spontaneous assembly in an aqueous solution (Scheme 1). This type of novel assemblies might be used as versatile nanocarriers, considering the excellent inclusion-solubilization performance of β -CD to many hydrophobic drugs.[8] In

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addition, this strategy circumvents the relatively complicated synthesis procedure frequently involved 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]

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) (Figure S1a). α -Methoxy- ω -amino-PEG ($M_w=5000$) was selected as a hydrophilic segment. A block copolymer (**1**) with a polyaspartamide block containing ethylenediamine (EDA) units (PEG-*b*-PEDA) was synthesized as reported previously.[10] ^1H NMR measurement indicates that the number of structure unit of PEDA block is about 12, which is consistent with the MAIDI-TOF result shown in Figure S2b. β -CD was then covalently linked to PEG-*b*-PEDA to obtain PEG-*b*-PCD (**2**). Tracing the reaction process through ^1H NMR indicates that temperature and reaction time are important factors to the conjugation of β -CD onto PEDA segment. Optimized reaction conditions, i.e. excess 6-monotosyl β -CD at 70°C for 5 days, were adopted to synthesize **2**. By doing this, a high efficient conjugation (up to 90%) can be achieved; this is especially true for copolymers with relatively short PEDA chains. As for copolymer mentioned in the following study, ^1H and ^{13}C NMR spectra are shown in Figure S1b. Calculation based on ^1H NMR spectrum suggests that about 10 β -CD molecules were introduced into the side chains of PEDA segment. To a certain degree, this agrees with MAIDI-TOF measurement (Figure S2c). The resultant 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 as evidenced by temperature dependent ^1H NMR spectra in DMSO- d_6 (Figure S3).

To investigate the formation of polymeric assemblies mediated by the host-guest interactions between PEG-*b*-PCD and hydrophobic substances, pyrene was initially used as a guest molecule. The normalized emission spectra of pyrene in aqueous solutions containing PEG-*b*-PCD are shown in Figure 1a. With the increase in PEG-*b*-PCD concentration, significant enhancement in excimer intensity (420–600 nm) can be observed. In the case of β -CD, however, no excimer formation can be observed (Figure S4). As well known, 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 pre-associated pyrene pairs in the ground-state.[11] For PEG-*b*-PCD, the excimer formation may likely be due to 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).[12] Additionally, the significant decrease in the excitation intensity of PEG-*b*-PCD solution is likely resulted from the quenching phenomenon, suggesting the existence of a local high concentration of pyrene. As shown in Figure S6, a significant bathochromic shift with the broadening in vibrational structure of pyrene excitation spectrum monitored at 475 nm compared with that monitored at 390 nm, also supports the ground-state dimer formation of pyrene.[12] Plots of concentration dependent changes in intensity ratios of I_{338}/I_{333} , I_3/I_1 and I_E/I_M are shown in Figure 1b. Significant increase 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 1b). No significant changes in I_E/I_M , however, were found in the case of β -CD (Figure S4b). 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 locate in a more hydrophobic microenvironment in the existence of 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, 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 containing pyrene (0.6 wt.%) were prepared by dialysis method. Height and 3D AFM images of the PEG-*b*-PCD assemblies containing pyrene are shown in Figure S7. These AFM images show assemblies of a round shape with diameters in the range of 20 to 120 nm. AFM sectional analysis (Figure S8a, for example) shows that the diameters of the assemblies are generally ten- to seventeen-times larger than the heights of the aggregates. This 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] In addition, the tip convolution effect could also be responsible for this phenomenon.[16] Calculation based on AFM images gives a mean size of 63.5 nm, while dynamic light scattering (DLS) measurement shows a mean diameter of 27.3 nm (Figure S9a). In addition, TEM images reveal the mean size is about 20.0 nm (Figure 2a), which is well consistent with the DLS result. A similar phenomenon of simultaneous encapsulation and assemblies formation was also observed for PEG-*b*-PCD in the presence of other hydrophobic compounds such as indomethacin (IND) and coumarin 102 (Figures S8b, S9b and S10). In addition, a preliminary *in vitro* release study was performed to demonstrate the chemical stimulated release behavior of assemblies based on PEG-*b*-PCD and IND, a non-steroidal anti-inflammatory drug. As shown in Figure 3, in the presence of adamantane-carboxylic acid (ADCA), a competition guest molecule with a higher complexation constant compared with that of IND, drug release rate was accelerated. This effect was gradually levelled off in the later release stage.

As a partial conclusion, above results suggest that core-shell aggregates can be assembled by PEG-*b*-PCD in an aqueous solution in the presence of a hydrophobic compound, a process mediated by host-guest interactions between 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 pseudo-amphiphilic block copolymer. In other words, the hydrophobic compounds hydrophobilize the PCD block of PEG-*b*-PCD copolymer. Further assembly of this pseudo-amphiphilic copolymer in aqueous solution may form the core-shell nano-assemblies. Additional free hydrophobic molecules can be simultaneously encapsulated in the cores of assemblies due to the hydrophobic interaction. This process can be schematically illustrated in Scheme 1a.

Now we turn our attention to the assembling behavior of PEG-*b*-PCD in the presence of a hydrophobic polymer. Previous study by Jiang's group shows the formation of micelle-like aggregates by a β -CD containing homopolymer and a polymer with an adamantyl side group. [17] In this study, poly(β -benzyl L-aspartate) (PBLA) with M_n of 2000 (Figure S2d) was selected as the model guest polymer. Assemblies based on PEG-*b*-PCD/PBLA were prepared by dialysis procedure using DMSO as a common organic solvent. As shown in Figure 2b, spherical assemblies with mean diameters ranging from 50 to 200 nm were obtained with a theoretical feed ratio of 1:20 (weight ratio of PBLA to PEG-*b*-PCD), and analysis based on TEM images indicates the mean size to be about 96.4 nm. This value agrees with that determined by DLS (118.7 nm) as shown in Figure S9c. On the other hand, observation by SEM indicates the mean size to be about 256.7 nm (Figure S11). 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 2c. The mean size determined by DLS was 209.2 nm (Figure S9d). The cores of these assemblies were investigated by ^1H NMR and fluorescence anisotropy. As shown in Figure S12, no signals corresponding to PBLA can be observed for PEG-*b*-PCD/PBLA assemblies in D_2O . However, signals at 7.3 and 5.0 ppm that are

characteristic peaks of protons corresponding to benzyl group, are evident in DMSO. This indicates that the cores of these assemblies are mainly comprised of PBLA. In addition, staining using phosphotungstic acid (PTA) enabled us to directly observe the core-shell structure of these assemblies. As shown in Figure 4b, 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 TEM images shows the average thickness of shells to be about 30 nm. This result suggests that the shells of 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 PEG block length. Further information on the microviscosity of inner core was provided by the depolarization of fluorescence using 1,6-diphenyl-1,3,5-hexatriene (DPH) as fluorophore. The anisotropy value of r measured for DPH in aqueous solution of PEG-*b*-PCD/PBLA assemblies is 0.2, while r is 0.25 for PEG-*b*-PBLA micelles, suggesting 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 system based on polymeric assemblies to be administered by systemic injection. In addition, lyophilized samples of PEG-*b*-CD/PBLA assemblies can be re-dispersed 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). Additionally, by using PEG-*b*-PCD/PBLA based assemblies as nanocarriers, sustained *in vitro* release of coumarin 102 was achieved as (see Figure S14).

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 iodine (KI) and benzyl alcohol (BA), were selected because both of them can form inclusion with β -CD.[18] As illustrated in Figures 4c and d, inter-particle aggregates were formed in the presence of either KI or BA. The morphologies were significantly different from those of original separate assemblies (Figure 4a). The chemical-triggered aggregation of the assemblies is likely due to the de-shelling effect resulting 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). This unique characteristic might be beneficial in drug delivery, considering the PEG dilemma in drug/gene delivery; 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 de-PEGylation.

Based on these results, we can conclude that well-defined core-shell assemblies can be successfully prepared by PEG-*b*-PCD copolymer 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 Scheme 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 developed by the groups of Kataoka and Kabanov, have attracted great attention due to their potential applications in biomedicine and pharmaceuticals.[2c,6][20] Accordingly, it is interesting to construct PIC assemblies by the host-guest interaction of PEG-*b*-PCD and guest molecules. For a preliminary study, ADCA was selected as a guest molecule, since the strong inclusion interaction of adamantyl group with β -CD has

been well demonstrated.[21] First, a pseudo-polyelectrolyte copolymer with one negatively charged block was 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 comprised of PEI and ADCA-inserted block of PEG-*b*-PCD (Scheme 1c). TEM observation indicated that these assemblies were spherical with a mean diameter of 100.7 nm (Figure 2d). The mean size determined by DLS was 97.1 nm (Figure S9f). These results demonstrate that PEG-*b*-PCD copolymers may be used to construct delivery vectors potentially for water-soluble macromolecules such as proteins, therapeutic DNAs and siRNAs.

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 complex with hydrophobic substances, while the hydrophilic segment can impart the resultant assemblies with stability. Host-guest recognition mediated nano-assemblies 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 using this type of copolymers. Based on the well established knowledge of the solubilization effect of various cyclodextrins (α , β or γ) to a broad range of hydrophobic compounds, cyclodextrins bearing block- or graft-like 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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(a)

(b)

Figure 1.

Normalized emission spectra of pyrene in aqueous solutions containing PEG-*b*-PCD. (a), emission spectra with an excitation wavelength at 339 nm; (b), plots of I_{338}/I_{333} , I_3/I_1 and I_E/I_M as a function of PEG-*b*-PCD concentration. 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; I_E/I_M -- the intensity ratio of the excimer (475 nm) to monomer (371 nm) in emission spectrum. [Pyrene] = 6.0×10^{-7} M.

Figure 2.
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.

Figure 3.

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

Figure 4. TEM images of assemblies based on PEG-*b*-PCD/PBLA of 15:6 (a) control, (b) staining with PTA, (c) in the presence of 0.05M KI, and (d) in the presence of 0.05M benzyl alcohol.

Scheme 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) polyion complex (PIC) like assemblies.