Bioconjugate Chemistry

Communication

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Camptothecin–Polysaccharide Co-assembly and Its Controlled Release

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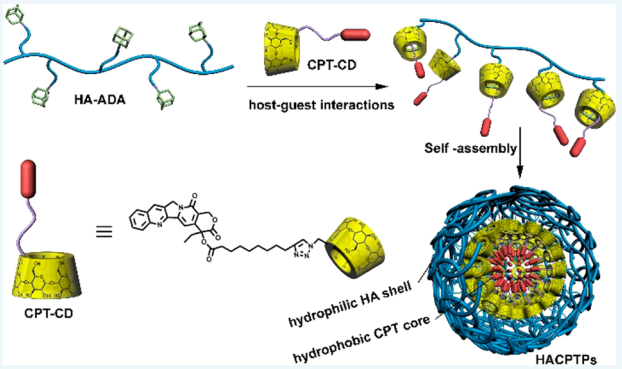
Supporting Information

ABSTRACT: β-Cyclodextrin modified camptothecin (CPT–CD) was synthesized through esterification reaction and "click chemistry" to greatly improve the solubility of CPT in aqueous solution, and then, a supramolecular nanoparticle was constructed by strong noncovalent interaction between β-cyclodextrin and adamantane and amphiphilic interaction by simply mixing CPT–CD and adamantane modified hyaluronic acid (HA–ADA) together. The obtained nanoparticle had a hydrophilic HA shell, which could target and recognize HA receptors overexpressed on the surface of cancer cells, and a hydrophobic CPT core, which could protect CPT from hydrolyzation. The results of cytotoxicity experiments showed that the nanoparticle we have designed in this work exhibited similar anticancer activities to, but with much lower side effects than, the commercial chemotherapeutic drug CPT in vitro. We believe that this work might provide a strategy for improving the treatment performance of CPT in laboratory and clinical settings.



The construction of complicated supramolecular nanostructures composed of micromolecules, polymers, or inorganic materials by simple noncovalent interactions has become a research hot-spot in recent decades,[1,2] most of the nanostructures having wide application prospects in numerous research fields, such as medicinal chemistry, material chemistry, catalytic chemistry, biochemistry, and so on.[3−5] In addition, the design and construction of novel targeting drug-delivery systems was recently begun. These systems exhibited satisfactory tumor inhibition effects and low side effects in vitro and in vivo,[6−10] overcoming the shortcomings of the traditional chemotherapeutic anticancer drugs with low aqueous solubility and high physical toxicity, which drew the broad attention of biochemists and medicinal scientists. For an advanced drug-delivery carrier, good aqueous solubility, synthetic convenience, high binding capability with anticancer drugs, good biocompatibility, biodegradability for drug release, and targeting effects determined the therapeutic efficiency of the drug-delivery system.[11] Currently, a variety of supramolecular drug-delivery systems with multitreatment based on inorganic nanoparticles,[12,13] supramolecular organic frames,[14−18] carbon nanomaterials,[19−22] micelles and vesicles,[23−28] and so on were tactfully designed and constructed, and they exhibited exciting cancer treatment effects. However, the interminable synthetic routes of the drug carrier, the low loading efficiency, the low aqueous solubility, and inactivation of the loaded anticancer drugs were still problems for the further clinical application of the targeting drug-delivery systems. Therefore, the clever design and careful choice of the carrier's materials and loaded drug became the emphasis for the novel advanced drug-delivery system.[29,30]

Among various drug carrier materials, hyaluronic acid (HA), a sort of polysaccharide with excellent aqueous solubility, biocompatibility, biodegradability by hyaluronidase, facile modification, and targeting capability toward HA receptors (CD44 and RHAMM receptors) overexpressed on the surface of malignant cancer cells, became a new generation of building blocks for nanosized particle construction for drug and gene delivery and cancer diagnosis.[31−36] For instance, Zhang, Huang, Dong, and co-workers[37] synthesized diiodostyryl bodipy conjugated hyaluronic acid, and this compound could self-assemble to form nanoparticles, which could target and assist in the diagnosis of tumors and suppress tumor growth effectively in photodynamic therapy way *in vitro* and *in vivo*. Our group[38] combined β-cyclodextrin modified hyaluronic acid, adamantane-bis(diamine) conjugate, and cucurbit[6]uril (CB[6]) together to construct triple-component nanoparticles, and the addition of CB[6] could protonate the diamine groups in adamantane-bis(diamine) conjugate effectively, which could bind and deliver

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negative charged siRNA into cancer cells to interrupt the expression of specific protein.

In this work, we utilized the reported adamantane modified HA (HA−ADA) as drug carrier and then synthesized the β-cyclodextrin modified camptothecin (CPT−CD) to improve the aqueous solubility of CPT. When the HA−ADA and CPT−CD were mixed together, taking advantage of the strong noncovalent interaction between adamantane and β-cyclodextrin and the supramolecular amphiphilic interaction, we obtained a kind of nanoparticle (HACPTPs), and the HACPTPs had hydrophilic HA shells, which could recognize HA receptors overexpressed on the surface of cancer cells, and hydrophobic CPT core, which could protect CPT from hydrolysis against water; this might result in the inactivation of CPT. The construction process of HACPTPs was illustrated in Scheme 1. In addition, the obtained nanoparticles HACPTPs exhibited similar cancer cell inhibition effects to that of commercial CPT but much lower cytotoxicity than that of CPT toward normal cells. This work might provide a protocol for solving the insoluble and untargeting problems of CPT.

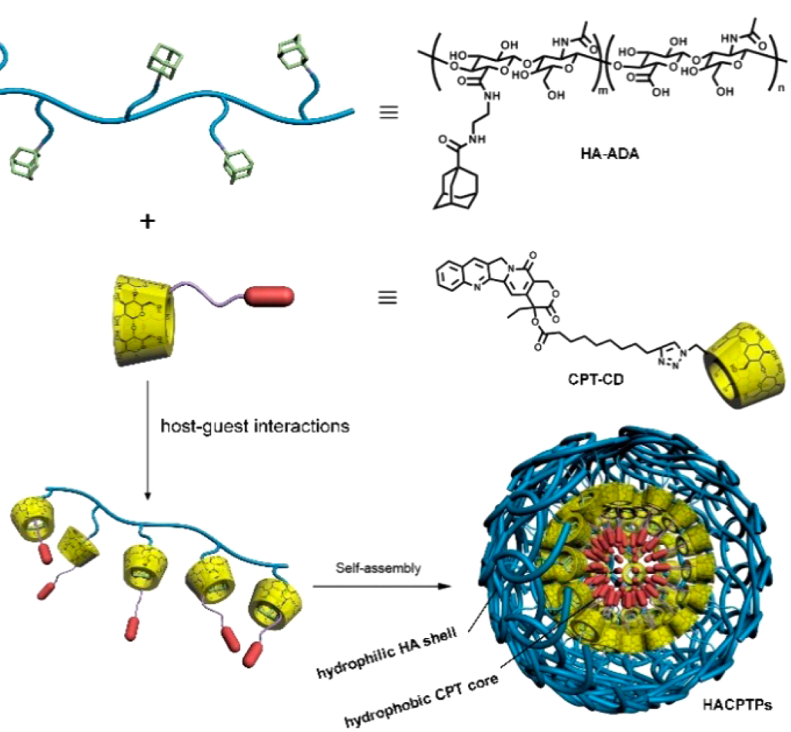
The synthetic routes of CPT−CD was illustrated in Figure S1. Alkynyl group was first appended onto CPT molecule by esterification reaction of CPT with 10-undecynoic acid, and then the 6-deoxy-6-azido-β-CD was connected with CPT by "click chemistry" in 63% yield. As shown in Figure S5, a typical proton signal of triazole ring appeared at 8.7 ppm, and the proton signals assigned to β-CD also showed around 3−4, 4.5, 4.8, and 5.5 ppm, which together verified the successful connection of β-CD with CPT. After that, the CPT−CD exhibited satisfactory aqueous solubility, which was measured as 3 mM in PBS containing 3% DMSO, which was much higher than that of CPT as 6 μM in water,[39] and the greatly improved aqueous solubility would facilitate the anticancer activity of CPT−CD in vitro and in vivo.

However, HA−ADA was prepared according to our previous work,[40] and the degree of substitution of HA−ADA was calculated as 13%, indicating that adamantane groups were grafting onto the backbone of HA every 7.7 repeating sugar units on average. This would not impede the targeting effects of HA toward CD44 and RHAMM receptors overexpressed on the surface of the cancer cells; these required at least six successive sugar units as one targeting section.[41]

To investigate the complexation behavior between CPT−CD and HA−ADA, we employed a synthetic intermediate ADA-EDA as a reference guest for ^{1}H NMR titration. As shown in Figure 1a, when the concentration of CPT−CD increased from 0 to 3.0 mM, the proton signals of ADA-EDA (0.5 mM) shifted downfield gradually accompanying shape changes, which indicated that the inclusion of ADA into the cavity of CPT−CD.[11,42] Moreover, by analyzing the nonlinear least-squares fit of the titration data (Figure 1b), the binding constant (K_S) between ADA−EDA and the β-CD cavity of CPT−CD was calculated as $(1.8 \pm 0.2) \times 10^3$ M^{-1}. In addition, the stoichiometry between ADA-EDA and CPT−CD was determined as 1:1 according to the Job's plot in which the maximum was observed at a molar fraction of 0.5 (Figure S9).

Taking advantage of the satisfactory interactions between adamantane and β-CD, a supramolecular nanoparticle (HACPTPs) composed of CPT−CD and HA−ADA was successfully constructed by simply mixing the two components mentioned above. The size and morphology of the nanoparticles were characterized by atomic force microscope (AFM), high-resolution transmission electron microscope (HR-TEM), dynamic light scattering (DLS), and ζ potential experiments. As shown in Figure 2a, a series of collapsed nanoparticles with spherical shape were observed in a typical AFM image, and the height of the nanoparticle was measured as 7.3 nm, which was basically equal to the sum of the values of two HA backbones

Scheme 1. Construction of HACPTPs nanoparticles



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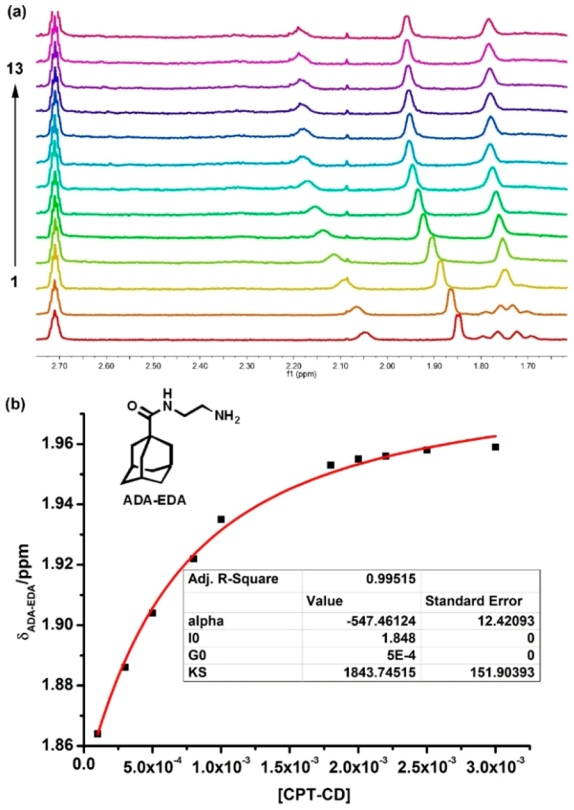

Figure 1. ^{1}H NMR titration of ADA-EDA with CPT−CD. (a) 1H NMR spectra of ADA-EDA (0.5 mM) upon the addition of 0, 0.1, 0.3, 0.5, 0.8, 1.0, 1.2, 1.5, 1.8, 2.0, 2.2, 2.5, and 3.0 mM CPT−CD (spectrum 1 to 13) in D_2O containing 3% DMSO-d^6 at 25 °C. (b) Nonlinear least-squares fit of the chemical-shift changes of the ADA-EDA peaks at δ = 1.86 ppm as a function of the concentration of CPT−CD.

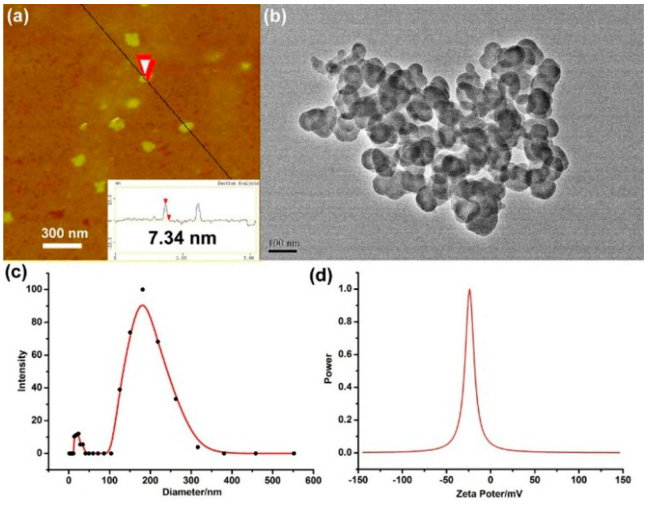

Figure 2. Typical (a) AFM and (b) HR-TEM images of HACPTPs and (c) DLS and (d) ζ potential experimental results of HACPTPs in PBS.

(*ca.* 1.8 nm), two cyclodextrins (*ca.* 1.7 nm), and two CPT molecules with hydrophobic undecynoic chain (ca. 4.0 nm). The HR-TEM image (Figure 2b) also showed the homogeneous spherical nanoparticles whose diameters distributed from 70 to 90 nm, and these nanoparticles tended to self-aggregate due to the hydrogen-bonding interaction among the HA hydrophilic shell of the HACPTPs nanoparticles. Moreover, the results of DLS experiments (Figure 2c) exhibited the hydrodynamic diameter of HACPTPs as ca. 118 nm, which was very close to the result of HR-TEM image (70−90 nm) and much larger than the hydrodynamic diameter of CPT−CD, ca. 80 nm (Figure S10), indicating the formation of the nanoparticles. Furthermore, the DLS results at different scattering angles showed that the gyration (R_g) and hydrodynamic (R_h) radii of HACPTPs were determined as 61.1 and 91.6 nm, respectively (Figures S11 and S12). Thus, the R_g/R_h value was calculated as 0.67, which was less than 0.78 (corresponding to the solid micellar nanoparticles), suggesting that the HACPTPs were micelles with core−shell structures, as illustrated in Scheme 1.[43] However, the surficial charge of HACPTPs was determined by ζ potential experiments. As shown in Figure 2d, the HACPTPs showed a typical surficial negative charge as ca. −24 mV due to the negative charged hydrophilic HA shell of the nanoparticle, and this negative charged surface would facilitate the stability, dispersibility, and biocompatibility of HACPTPs in biological environments and prolong the circulation time in vivo.[11,44] The control experiment showed that the CPT−CD was uncharged at the surface, and the ζ potential was measured as ca. −0.04 mV (Figure S13).

Furthermore, the successful construction of HACPTPs nanoparticles could be also distinguished by naked eyes. As shown in Figure S14a, HACPTP nanoparticles could exist as steady and transparent aqueous solution and exhibited much more obvious Tyndall effects than did the HA−ADA polymer, which meant the formation of nanoscaled particles. Moreover, after dialysis against deionized water, the HACPTPs nanoparticles in PBS still emitted typical blue fluorescence of CPT, and no precipitation was observed (Figure S14b), which further proved the formation and stability of the HACPTPs nanoparticles.

Next, we performed cytotoxicity experiments to evaluate the anticancer activities of HACPTPs nanoparticles. As shown in Figure 3a, CPT dissolved in DMSO exhibited satisfactory malignant cell inhibition effect toward HA receptor positive HCT-116 human colon cancer cells, whose relative cellular viability was measured as 22%. After the grafting of β-CD, the CPT−CD showed remarkable aqueous solubility; however, the relative viability of cancer cells was 35%, which was little higher than that of CPT, which might be attributed to the slight anticancer activity decline caused by the modification of CPT by β-CD. However, after forming supramolecular nanoparticles between CPT−CD and HA−ADA, the obtained HACPTPs exhibited similar anticancer activity to that of CPT, and the relative cellular activity was obtained as 22%. However, after adding an excess of 10 equiv of HA, the cancer cell inhibition of HACPTPs decreased distinctly, and the relative cellular viability was measured as 55%. This phenomenon indicated that the interaction of HA and the HA receptor plays an important role in the internalization of HACPTPs into cancer cells.

Moreover, the side effects of HACPTPs was also evaluated by using HA receptor negative NIH3T3 mouse embryonic fibroblasts. As shown in Figure 3b, both CPT and CPT−CD exhibited cellular damage effect and gave relative cellular viabilities of 17% and 38%, respectively. Due to the lack of HA receptor on the surface of normal cells, HACPTP nanoparticles could be hardly internalized into cytoplasm of NIH3T3 cells and showed the high cellular viability as 86%. Furthermore, the carrier HA−ADA showed no cytotoxicity to both of cancer cells

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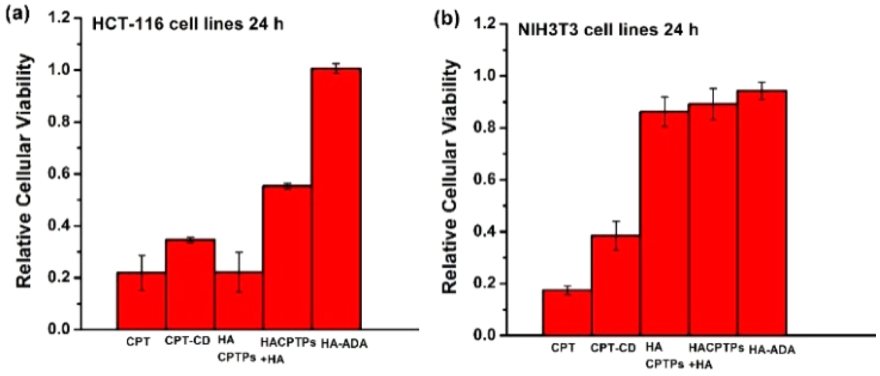

Figure 3. Cytotoxicity experiments results of (a) HCT-116 cells and (b) NIH3T3 cells in 24 h.

and normal cells, and the results of cytotoxicity experiments in 48 h (Figure S15) were basically consistent with the ones in 24 h. Finally, by these phenomenon, we could deduce that the HACPTPs could enter into cancer cells via the HA receptor mediated endocytosis effect and showed a similar anticancer effect with commercial CPT but much lower side effects in normal cells compared to those for CPT.

In conclusion, we successfully synthesized the β-CD-modified CPT through esterification and "click chemistry", and the compound exhibited satisfactory aqueous solubility to solve the problem of insolubility of CPT in most of organic and aqueous solvents. Next, by taking advantage of the strong supramolecular interactions between β-CD and adamantane, supramolecular nanoparticle HACPTPs composed of CPT−CD and HA−ADA was constructed by host−guest interaction and amphiphilic interaction. The obtained HACPTPs had a hydrophilic and biocompatible HA shell, which could recognize and target HA receptor overexpressed cancer cells, and a hydrophobic CPT core, which could prevent CPT from hydrolysis and then inactivation. Finally, the results of cytotoxicity experiments proved that HACPTPs could be internalized into cancer cells by HA receptor mediated endocytosis and exhibited similar anticancer effects with but much lower side effects than commercial CPT. This work might provide a novel strategy, through which, due to simple and high-yield chemical modification and the convenient supramolecular assembly method, the insoluble chemotherapeutic agents could be loaded into aqueous nanoparticles or nanoplatforms with cancer cell-targeting capability and biodegradability. This might tremendously increase the anticancer activity and decrease the side effects of the anticancer drugs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.6b00606.

Experimental details, compound characterization, an image of Job's plot, spectra analysis results, the results of DLS and ζ potential analysis of CPT−CD, the Tyndall and fluorescent images of HA−ADA and HACPTPs, and the results of the cytotoxicity experiments for 48 h. (PDF)

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Notes

The authors declare no competing financial interest.

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