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The Core-Inversible Micelles for Hydrophilic Drug Delivery

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Abstract

A unique core-inversible micelle (CIM) was formed via PEG^{5k}CA₈ for hydrophilic drug delivery. An amyloid-fibril-inhibiting water-soluble molecule, congo red (CR), has been loaded into the hydrophilic core of CIMs. The targeting folate-CIMs significantly enhanced the intracellular delivery of hydrophilic CR in a folate receptor-expressing cell line.

Many diseases arise from the dysfunction of intracellular molecules. Therefore, intracellular delivery of therapeutics is critical in restoring the cellular functions, thus treating these diseases. Protein, peptide and gene therapeutics have been applied in the treatment of many diseases, including cancer. However, the therapeutic indexes of these hydrophilic reagents, including water-soluble small molecules, are limited by their poor permeability through the plasma membrane. In addition, hydrophilic molecules are unlikely drug candidates, since they may exhibit unfavourable pharmacokinetic profiles in terms of the absorption, distribution, metabolism and excretion (ADME). Conjugation of cell-targeting proteins¹ or cell penetrating peptides (CPP)² have been applied for the intracellular delivery of hydrophilic cell-membrane-impermeable peptides and proteins. At the same time, synthetic cationic nanocarriers³ have been developed for delivery of peptides/proteins^{3a} and gene molecules^{3b}. But the off-target toxicity of the CPP and cationic synthetic nanocarriers, as well as the immunogenicity of immunotoxins^{3b} hampered their systemic administration. Strategies for the systemic delivery of hydrophilic molecules,⁴ e.g. PEGylation,^{4a} polymer nano/microsphere^{4b} and liposomes^{4c} have been developed. However, novel nanocarriers are desired for the intracellular delivery of water-soluble drug molecules.

Cholic acid (CA) is a primary bile acid secreted into GI track for the digestion of fatty nutrients. The facial amphiphilic structure of CA has attracted a broad interest in the field of supramolecular chemistry⁵ and polymer chemistry⁶. CA-containing molecular devices have been developed as molecular cages⁵ for the encapsulation of guest molecules or served as an artificial ion channels⁷ or vehicles⁸ for the trans-membrane transportation of hydrophilic

molecules. However, these oligo-CA constructors usually have poor water solubility and small cavities, therefore limiting their application for drug delivery.

We have developed a novel biocompatible linear-dendritic amphiphilic co-polymer, i.e. PEG^{5k}CA₈ shown in Figure S-1, composed of polyethylene glycol (PEG 5000) and a dendritic octamer of cholic acid (CA₈).⁹ It self-assembles into micelles in aqueous solution for efficient delivery of hydrophobic drugs, such as paclitaxel (PTX),^{9b} doxorubicin (DOX)^{9d} and vincristine^{9c} etc. Interestingly, PEG^{5k}CA₈ were found to self-assemble selectively in certain apolar solvents into core-inversible micelles (CIMs) with a PEG shell and a hydrophilic core, driven by the hydrogen bonding (Figure S-2).

The particle sizes of PEG^{5k}CA₈ aggregates in different solvents were measured by dynamic light scattering (DLS). The particle sizes (Mean $\pm$ SD, based on area distribution) are listed in descending order of the solvent polarity: water (18.6 ± 3.4 nm) > acetonitrile (21.8 ± 3.9 nm) > acetone (15.1 ± 2.7 nm) ~ ethanol (1.1 ± 0.2 nm) > THF (1.6 ± 0.1 nm) > DCM (binary peaks, 2.2 nm and 10.0 nm) ~ ethyl acetate (15.9 ± 2.8 nm) > toluene (24.5 ± 4.6 nm) > CCl₄ (36.9 ± 7.0 nm) (Figure S-3). PEG chain in telodendrimer is soluble in all the solvents selected above. However, oligo-CA in telodendrimer selectively aggregates in different solvents, due to the relative solvophobicity of two distinct surfaces of CA.¹⁰ It is evident that PEG^{5k}CA₈ self-assembles into nanoparticles in the solvents with either high polarity (water, acetonitrile and acetone) or relative low polarity (ethyl acetate, toluene and CCl₄) driven by the hydrophobic or hydrophilic interactions (e.g. H-bonding), respectively. In the solvents with medium polarity with strong H-bonding capability, such as THF and ethanol, the hydrophobic interactions between CA motifs are diminished and H-bonds are saturated by solvent molecules,¹¹ which prevent the aggregation of telodendrimers.

PEG proton NMR signals in PEG^{5k}CA₈ at 3.5-3.6 ppm were detected in all solvents used in this study (Figure S-4), indicating that the PEG layer was fully solvated. In contrast, proton signals of the CA structure were completely undetectable in D₂O, indicating the collapse of CA into the core of the micelles.^{9a, 12} The suppression of the NMR signals on CA was also observed in nonpolar toluene and CCl₄. TEM images (Figure 1) also revealed the spherical CIMs in these apolar solvents. On the contrary, no CIM nanoparticle was observed in DMSO (Figure S-5) under TEM and a well-defined proton NMR spectrum of PEG^{5k}CA₈ was recorded in DMSO (Figure S-4), both indicating the complete solvation of PEG^{5k}CA₈ in DMSO. In chloroform and acetonitrile, significant broadening of CA proton signals was observed, indicating that certain entanglements of oligo-CA occurred. The TEM image revealed the spherical nanoparticles of CIMs in acetonitrile, however with unclear edges, indicating loose aggregations (Figure S-5).

Given the CIM formation in apolar solvents, we would like to probe the polarity of the core in CIM. 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) is a water-soluble fluorescent molecule with the distinct shift in UV-Vis absorbance and emissions upon deprotonation respectively (Figure S-6).¹⁰ As shown in Figure 2A, the deprotonated HPTS (pH 10) was loaded in PEG^{5k}CA₈ CIMs via a freeze-dry method (illustrated in Figure S-2) in five different solvents, namely, toluene, ethyl acetate, ethanol, acetonitrile and water. Their particle sizes were measured from 14.7 nm to 26.1 nm in these solvents except in

ethanol, where the only oligomer of PEG^{5k}CA₈ was observed at 5 nm. The blue fluorescence of HPTS in ethanol indicated a low polar environment in ethanol, prohibiting HPTS deprotonation. In water and acetonitrile, the protonated HPTS directly dissolved in the bulky solvents, showing green fluorescence. The green and blue-green fluorescence of HPTS in apolar toluene and ethyl acetate in the presence of CIMs indicated that HPTS was surrounded by a relatively high polar microenvironment, which promoted HPTS deprotonation. In addition, the absorbance of the deprotonated HPTS at 450 nm increased with a red-shift after being loaded into the CIMs in apolar solvents, such as toluene and ethyl acetate (Figure 2B). Coincident with the above fluorescence study, a small peak at 475 nm was observed in ethanol and was exhibited as a major peak in acetonitrile. Surprisingly, the HPTS-O⁻ peak at 450 nm was relatively small in aqueous micellar solution, which may be due to the encapsulation of HPTS into the hydrophobic core of the nanocarrier, prohibiting HPTS deprotonation.

The reverse micelles (RMs), formed by small molecular surfactants or some amphiphilic block copolymers,¹³ have the complete core-shell inversed structures in apolar solvents compared with their normal micelles in water. Differently, CIMs share the same PEG shell with their normal micelles in water as illustrated in Figure S-2. This makes it possible to transport the drug-loaded CIMs from organic solvent into aqueous solution via a simple aqueous extraction, owing to the dominant partition of PEG in water. Based on this unique phenomenon, we are exploring the applications of CIMs in encapsulating hydrophilic bioactive molecules for intracellular drug delivery. Congo red (CR), an ionic water-soluble dye (Figure 3A), has been used to stain amyloid fibrils formation. Furthermore, it has been reported to inhibit the amyloid oligomerization to treat neurodegenerative diseases.¹⁴ However, the incapability of CR to cross blood-brain barrier (BBB) limited the efficacy of CR in Huntington's disease mouse models,^{14c, 15} which may be addressed via the nanoparticle-based drug delivery.¹⁵

The encapsulation of CR into CIMs significantly increases its solubility in ethyl acetate, whereas CR is completely insoluble. The particle size of the CR loaded CIMs in ethyl acetate was measured via DLS to be 14.4 ± 3.4 nm (Figure S-7). As discussed above, the CIMs loaded with CR could be easily transferred into aqueous phase from ethyl acetate via simple extraction with mild shaking for minutes or standing for hours (Figure 3B). The completion of CIMs extraction was further confirmed via a FITC-labeled fluorescent micelle (Figure S-8). After being loaded into CIMs, a red shift of CR absorbance from 500 nm to 515 nm was observed, which indicates the interactions of CR with CIMs (Figure S-9). At the same time, the fluorescence intensity of CR was dramatically enhanced after being loaded into CIMs in ethyl acetate as well as after aqueous extraction (Figure 3C). Almost identical fluorescent spectra of CR-CIMs before and after extraction indicated that CR was loaded in an intact microenvironment during aqueous transfer. The restrictions of the intermolecular rotations (RIR) significantly increase the quantum yield and turn on the emission of CR molecules.¹⁶ Compared with free CR, the aqueous extraction of the CR-loaded CIMs was eluted much faster in a gravity size exclusive chromatograph (SEC), showing that CR molecules were encapsulated in nanoparticles (Figure 3D). The content of CR in CIMs was detected via UV-Vis absorbance to be 1% (w/w, CR to CIMs) with 100% of encapsulation efficiency. Furthermore, the CR loading procedure was optimized using 95% ethanol

aqueous solution to dissolve CR and PEG^{5k}CA₈ directly, followed by rotoevaporation and dispersion in EtOAc. This significantly increased the solubility and the loading capacity of CR in CIMs (12.7%, CR/CIMs, w/w) with over 83% encapsulation efficiency after aqueous extraction.

Furthermore, the CR release from CR-CIMs in aqueous solution was studied using dialysis methods and compared with the free CR aqueous solution. As shown in Figure 3E, the free CR diffused completely through the dialysis membrane (3,500 MWCO) within 8 hours; however, the CR released from CR-CIMs in aqueous solution much slower with 50% release on day 8 and 80% release on day 40, respectively. The sustained encapsulation of CR in CIMs may be due to the unique amphiphilic structure of cholic acid inside the core of CIM, which provides both hydrogen bonding and hydrophobic interactions with CR molecules.

Furthermore, CR was loaded into the CIMs decorated with folic acid (FA) on the surface as a targeting ligand to enhance the cell uptake of the nanoparticles. A folic acid receptor overexpressing colon cancer cell line, HT-29, was incubated with free CR, CR-CIMs and targeting CR-FA-CIMs, respectively, for 3 hours before being imaged under a fluorescence microscope. As shown in Figure 4 free CR only showed very weak staining on a small population of cells. Slight improvement of cell staining was observed for the CR encapsulated in the non-targeting CIMs. In contrast, the targeting nanocarriers FA-CIMs significantly increased the cell uptake of the fluorescent CR in HT-29 cells, which proved the concept of the intracellular delivery of the hydrophilic molecules via CIMs. It will be interesting to further test the *in vivo* efficacy of the targeting CR-CIMs in the degenerative mouse models.

In conclusion, we have demonstrated the formation of CIMs possessing hydrophilic core structures in some apolar organic solvents. Hydrophilic fluorescence probe molecules and hydrophilic bioactive molecules, e.g. HPTS and CR, have been loaded into these CIMs in apolar solvents with high efficiency. The folic acid-decorated targeting CIMs significantly enhanced the intracellular delivery of hydrophilic bioactive CR molecules. The efficient transfer of CIMs into aqueous solution with the hydrophilic payloads encapsulated is key and strongly suggests the application of CIMs for the *in vivo* delivery of hydrophilic therapeutics. Furthermore, we have developed a photo-crosslinkable CIM in organic solvents, which will be able to cage a broad range of hydrophilic payloads more efficiently after the aqueous extraction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Negatively stained TEM images of PEG^{5K}CA₈ in ethyl acetate and toluene. The right corners of the images were shown by the black-white color inversion with the same magnification.

Figure 2.

(A) The deprotonated HPTS (50 μ M) was encapsulated in CIMs in different solvents exhibiting different particle sizes and fluorescent colors at excitation of 365 nm. (B) The UV-Vis absorbance of the protonated HPTS at 450 nm was enhanced after being loaded in CIMs in apolar solvents.

Figure 3.

(A) The structure of ionic hydrophilic CR; (B) The picture showed the complete aqueous transfer of CR-CIMs from organic ethyl acetate via simple extraction; (C) The enhanced Fluorescence spectrum of CR after being loaded in CIMs in EtOAc and after being extracted into the aqueous solution (with the same volume); (D) The gravity SEC indicated that CR was loaded in CIMs after being transferred into aqueous solution, evidenced by a early eluted fraction; (E) Significant prolonged release profile of CR from CR-loaded in CIMs compared with free CR in aqueous solution.

Figure 4. Folate receptor-positive HT-29 cells were incubated with free CR, CR-CIMs and CR-FA-CIMs. The fluorescence images indicated that targeting nanocarrier, FA-CIMs, significantly increased the cell uptake of CR in HT-29 cells.