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Biological deoxycholic acid-coumarin conjugates: photo-switchable structures and self-assembly morphology

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ABSTRACT

Construction of functional materials and supramolecular chemistry systems using biological molecules has been paid considerable attention. In this letter, a deoxycholic acid-coumarin (**DAC**) conjugate with both segments originated from biological molecules was developed. The photo-induced structure transformation was thoroughly investigated. This method can provide a simple and highly-efficient strategy to construct complex bile acid derivatives. With the amphiphilic rigid steroidal skeleton, the **DAC** conjugates and their corresponding dimers exhibit excellent self-assembly properties, which can also be conveniently adjusted by the photo-induced conversion process. Interestingly, both hydrophilic and hydrophobic binding cavities were demonstrated existence in the assemblies, leading to the possibility to introduce various functional molecules to the systems simultaneously. Additionally, the fluorescence intensity of **DAC** can also be adjusted with the structure conversion. Based on these properties, this biological originated molecule can be used as controlled-release materials and have the potential to observe the real-time condition in living cells.

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Biological molecules are particularly interesting as building blocks to construct new functional materials and supramolecular systems due to their superiority when employed for medical purposes as sensors, drug carriers, and scaffolds in tissue engineering etc.^[1] Bile acids are naturally occurring amphiphilic compounds and play important roles in a series of biological processes, such as the solubilization of fats in living organisms.^[2] Different from the conventional amphiphilic molecules, bile acids possess a steroidal skeleton with a concave hydrophilic face and a convex hydrophobic surface.^[3] Based on the rigid structure and unique distribution of hydrophobic and hydrophilic regions, bile acids have been demonstrated as powerful building blocks in constructing a wide variety of functional chemical systems and supramolecular architectures.^[4]

Coumarins, as another class of bio-resourced molecules, have attracted considerable attentions in a wide variety of research fields such as biology, medicine, and polymer science.^[5] With the properties of reversible photodimerization ($\lambda > 300$ nm) and photocleavage ($\lambda < 260$ nm) under UV irradiation, they have also been widely investigated in the application of light response materials.^[6]

Combination of these two biological originated molecules, a new deoxycholic acid-coumarin (**DAC**) conjugates were developed by simple amidation reaction. **DAC** can retain the

Scheme 1 Photo-induced structure conversion from **DAC** to **DACD**.

properties of both deoxycholic acid (**DA**) and coumarin segments. Under UV irradiation, the **DAC** structure undergo reversible photodimerization and photocleavage (**Scheme 1**), which has been well investigated by ¹H NMR, ESI-MS, Uv-vis and fluorescence spectrum. With the procedure of molecular structure switchable, the conversion of self-assembly morphology from nanorods to nanospheres was also observed. Due to the curved amphiphilic skeleton of **DA**, the assemblies contain both hydrophilic and hydrophobic pockets, which have the ability to encapsulate various functional molecules simultaneously. The controlled morphology conversion can provide a useful means to tune the release of the capsulated molecules. Moreover, with the molecular photocleaved to monomers, the fluorescence intensity of **DAC** was recovered, which could be used to image the photo-controlled drug release procedure in living cells.

DAC derivatives were prepared according to procedure reported by our group previously (**Scheme S1**).^[7] **DAC** was fully characterized by ¹H NMR, ¹³C NMR and ESI-MS spectrum (**Figure S1, S2**). It is well demonstrated by ¹H NMR spectrum

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Figure 1 Partial ^1H NMR spectrum (500 MHz, DMSO, 25 $^{\circ}\text{C}$) of DAC solution irradiated with 365 nm UV light, (a) 0 min, (b) 30 min, (c) 60 min, (d) 80 min, (e) 100 min.

that DAC exhibits the characteristic signals corresponding to the coumarin ring (Figure S1, Scheme 1): two doublets at 6.47 and 7.35 ppm are assigned to H_f and H_d respectively. Additionally, two doublets overlapped at 8.06–8.08 ppm belong to the signal of H_b and H_c . Whereas the signal of proton H_e is a doublet-doublet peak at 7.65 ppm owing to the coupling effect of H_d and the long-range coupling effect of H_b , which appears like a quartet. The signal of amide hydrogen H_a was appeared at 10.08 ppm by using DMSO- d_6 as NMR solvent.

Upon UV irradiation ($\lambda > 300$ nm), the DAC monomers can undergo photochemical dimerization through a [2+2] cycloaddition of two adjacent coumarin moieties.^[8] The photodimerization behavior of DAC was confirmed using ^1H NMR spectrum. Irradiation of DAC in DMSO- d_6 (2 mg mL^{-1}) with 365 nm UV light (24 W) can lead to the photodimerization of two neighboring coumarin groups and generate stable cyclobutane-based dimers (DACD). Before irradiation, there only appears one set of signals (Figure 1a). While after irradiation for 30 min (Figure 1b), two sets of signals corresponding to the original DAC and produced cyclobutane-based DACD were observed. The new resonances at 3.70–3.90 (H_e , H_f), 7.08 (H_d), and 7.50–7.58 (H_c , H_b) ppm were assigned to the dimerized coumarin protons. For the signal of amide proton H_a , a slightly upfield shift from 10.09 to 10.00 ppm was also observed. After irradiation for 60 min (Figure 1c), the integral area of peak H_d at 7.35 ppm decreased from 1.00 to 0.08 as compared to the original state. And the photoconversion ratio of the dimerization process was calculated to be 92%. When DAC was irradiated for 80 min (Figure 1d), only trace peak residue of DAC could be observed in the ^1H NMR spectrum, which can completely disappeared after 20 min longer irradiation (Figure 1e). This means that the photoconversion ratio of DAC can reach up to 100%. It is noted that in our system, DAC can be photodimerized completely in relatively short time without additional conditions, such as the confined space of γ -cyclodextrin.^[9] This may be due to the ordered arrangement of DA group in DMSO which may reduce the distance between two coumarin groups and thus promoting the photo-induced dimerization procedure.^[10] Conversely, this photodimerization can be reversed when DACD were irradiated by 254 nm UV light (28 W), which could be observed by the ^1H NMR spectrum changes (Figure S3). After 60 min of irradiation, about 80% of dimers were transformed to monomers (Figure S3d). Although

Figure 2 The absorption (A) and fluorescence (B) spectrum changes of DAC in DMSO solution on irradiation at 365 nm, 1×10^{-4} M, 25 $^{\circ}\text{C}$.

the irradiation time was elongated to 12 h, the photocleavage is still incomplete.

The photo-induced conversion can also be convinced by the mass spectrum. Before irradiation, a peak at m/z 571.9 belonging to $[\text{M}+\text{Cl}]^+$ is found in Figure S4a. After irradiation for 100 min, the peak of DAC monomer disappears with only the presence of $[\text{2M}+\text{Cl}]^+$ at m/z 1107.5 (Figure S4b), further verifying the structure conversion of DAC molecules.

To further study the photoconversion process of DAC in DMSO solvent, the characteristic absorption and emission spectrum of DAC were also monitored via UV-vis and fluorescence spectrometer. Upon increasing the irradiation time with 365 nm UV light, the characteristic absorption bands at 344 nm, which is ascribed to an $n-\pi^*$ transition related to the carbonyl function, was decreased gradually and finally disappeared when irradiated for 100 min, indicating the occurrence of coumarin photo-dimerization (Figure 2A).^[11] Figure S5 displayed very rapid reaction kinetics, where photo-dimerization degree reaches up to 95% within 60 min of irradiation at UV $\lambda=365$ nm. This is also in accordance with the result of ^1H NMR spectrum. While at higher energies there is a strong $\pi-\pi^*$ transition band at 260 nm corresponding to the conjugated π system, which showed continuously intensity decreasing.^[5h] The absorption spectrum changes may be due to the length of the conjugated π system is reduced after the photo-induced dimerization of coumarin moieties. Correlating with the changes of absorption spectrum, a decrease of the fluorescence emission band at 445 nm further indicating the photodimerization of coumarin groups (Figure 2B). Figure 2 shows the characteristic absorption spectrum as well as the fluorescence spectrum of DAC in DMSO solvent (1×10^{-4} M). Conversely, irradiation with UV light of 254 nm triggers the photocleavage of DACD and results in the recovery of the absorption band at 344 nm and of the emission band at 445 nm, which is due to the recovery of the extended conjugated π system (Figure S6, S7). The decrease in the dimerization degree is also shown in Figure S8. As revealed by the UV-vis and fluorescence spectrum, the photocleavage of DACD is incomplete due to the photo saturation and about 20% dimers remained after 1 h of irradiation. It is noted that accompanying the photo-induced

Figure 3 The TEM images of DAC (A) and DACD (B) in DMSO/ H_2O systems (1 mg mL^{-1}).

Figure 4 Comparison of the fluorescence emission spectrum of hydrophobic TPE probe (A) and hydrophilic ThT probe (B) in the absence and presence of **DACD** nanospheres; $\lambda_{\text{ex}} = 367$ nm for TPE and $\lambda_{\text{ex}} = 338$ nm for ThT.

structure conversion, the fluorescence emission of **DAC** almost “turned off” as **DACD** was obtained. Conversely, the fluorescence emission can also be “turned on” by 254 nm UV light irradiation. This controlled emission intensity transform may be useful in the cell imaging field.

We further investigated the self-assembly properties of **DAC** conjugates and their corresponding **DACD** dimers. With the amphiphilic **DA** skeleton and coumarin moieties, both molecules exhibit excellent self-assembly properties. As adding poor solvent (H_2O) dropwisely into the solution of **DAC** in DMSO (2×10^{-4} M, $V_{\text{DMSO}}:V_{\text{H}_2\text{O}}=2:1$), the Tyndall effect emerged and revealed that the nanometer level assemblies were generated. The assembly morphologies were visualized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The results in Figure 3A and Figure S9 clearly revealed that the assemblies of **DAC** have rod morphologies with several micrometers in length and average ~ 100 nm in diameter. It is worth noting that these nanorods possess an excellent rigidity, which may be due to the appearance of rigid steroidal skeleton. As the self-assembly of **DAC**, the corresponding UV-vis spectrum showed about 8 nm shift to 252 nm, revealing the H-aggregates of coumarin moieties in **DAC** assemblies^[12] (Figure S11). After the photo-induced dimerization of **DAC**, the assembly morphology exhibit significant changes from nanorods to nanospheres under the similar assembly conditions to that of **DAC** monomers. Both SEM and TEM images (Figure 3B, S10) showed that the **DACD** dimers can form well-dispersed nanospheres with average 200 nm in diameter. Because of the breaking of the conjugated π system of coumarins by photodimerization, the UV-vis spectrum of **DACD** did not show obvious changes after the assembly (Figure S12). The conversion of assembly morphologies further convinced the photo-induced monomer-dimer transformation. Furthermore, this photo-induced morphology conversion can also be used in the controlled drug delivery systems.

Li and coworkers have demonstrated the co-existence of the hydrophilic and hydrophobic binding sites in the assembled vesicles of bile acid derivatives.^[4g] In this work, we speculated that the hydrophilic and hydrophobic pockets still can co-exist in the **DACD** nanospheres. By using hydrophobic tetraphenylethylene derivatives (TPE)^[13] and hydrophilic thioflavin T (ThT),^[14] which are always used as probes to testify the existence of hydrophobic and hydrophilic microenvironments. Fig. 4A shows the fluorescence change of TPE in the absence and presence of the **DACD** nanospheres. Owing to the nonradiative energy decay arising from the free rotations of molecular bonds, the diluted TPE solution shows only diminished emission, but exhibits significant enhancement of 472 nm fluorescence in the presence of the **DACD** nanospheres. It is suggested that the existence of hydrophobic microenvironments to accommodate TPE molecules, and thus

restrict the rotational freedom of TPE molecule, which is responsible for the observed

Figure 5 Cell viability after exposure to **DAC** and **DACD** for 24 h.

Figure 6 CLSM images of HeLa 229 incubated with $10 \mu\text{g mL}^{-1}$ of **DAC** for 3 h. (a) Bright field, (b) excited with 440 nm laser, and (c) merged image of a and b.

fluorescence enhancement. Similarly, experiments with a hydrophilic ThT probe demonstrated the same enhancement phenomenon (Fig. 4B), and verified the existence of hydrophilic pockets in our **DACD** nanospheres. These results imply that these fluorescent nanospheres with embedded amphiphilic binding sites will have the potential to encapsulate different functional molecules. Because of the photo-induced morphology conversion, the controlled released of encapsulated molecules can also be achieved in our systems.

The cytotoxicity of **DAC** and **DACD** toward HeLa 229 was also established by using MTT viability assay. The results revealed that both **DAC** and **DACD** exhibit excellent biological compatibility and over 93% of the cells remained viable after incubating with $10 \mu\text{M}$ of **DAC** and **DACD** for 24 h (Figure 5). And extending the incubation time to 72 h did not show obvious effect to the viability of HeLa 229 (Figure S13). As revealed by the fluorescence spectrum, the emission intensity of **DAC** was gradually recovered as the photocleavage of **DACD** dimers. So in the living cell systems, as the photo-induced conversion of molecular structures from dimers to monomers, the imaging of cells under a laser scanning confocal microscope can also be recovered (Figure 6, S14). This biocompatible conjugates with self-assembly properties have the potential to be used as controlled drug-release materials and have the ability to detect the effect of drugs in the living cells by using a laser scanning confocal microscope.

In conclusion, we present here a **DAC** conjugates originated from biological sources and thoroughly investigated their photo-induced monomer-dimer conversion. Accompanying the structure transform, the self-assembly morphology also converted from nanorods to nanospheres. With the arched amphiphilic steroidal **DA** skeleton, the assembled nanospheres contain hydrophilic and hydrophobic pockets simultaneously, which can encapsulate both hydrophilic and hydrophobic functional molecules at the same time. And the controlled release of encapsulated molecules can be induced by the photo-induced morphology conversion.

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