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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 originating 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 to exist in the assemblies, leading to the possibility to introduce various functional molecules into the systems simultaneously. Additionally, the fluorescence intensity of **DAC** can also be adjusted with the structure conversion. Based on these properties, this biologically originated molecule can be used as controlled-release materials and has the potential to observe the real-time condition in living cells.

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 attention 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]

As a combination of these two biologically originated molecules, new deoxycholic acid–coumarin (**DAC**) conjugates were developed by simple amidation reaction. **DAC** can retain the 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 ^{1}H 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 dimers 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 ^{1}H NMR, ^{13}C NMR, and ESI-MS spectrum (Figs. S1 and S2). It is well demonstrated by ^{1}H NMR spectrum that **DAC** exhibits the characteristic signals corresponding to the coumarin ring (Fig. S1, Scheme 1): two doublets at 6.47 and 7.35 ppm are assigned to H_f and H_d respectively. Additionally, two doublets that overlapped at 8.06–8.08 ppm belong to the signal of H_b and H_e. Whereas the signal of proton H_c is a doublet–doublet peak at 7.65 ppm owing to the coupling effect of H_d and the long-range coupling effect of

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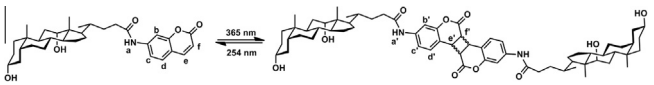

Scheme 1. Photo-induced structure conversion from **DAC** to **DACD**.

H_b, which appears like a quartet. The signal of amide hydrogen H_a 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 ^{1}H 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 (Fig. 1a), while after irradiation for 30 min (Fig. 1b), two sets of signals corresponding to the original **DAC** and produced by 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 (Fig. 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 (Fig. 1d), only a trace peak residue of **DAC** could be observed in the ^{1}H NMR spectrum, which can completely disappear after 20 min longer irradiation (Fig. 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 a relatively short time without additional conditions, such as the confined space of γ-cyclodextrin.[9] This may be due to the ordered arrangement of the **DA** group in DMSO which may reduce the distance between two coumarin groups 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 ^{1}H NMR spectrum changes (Fig. S3). After 60 min of irradiation, about 80% of dimers were transformed to monomers (Fig. S3d). Although the irradiation time was elongated to 12 h, the photocleavage is still incomplete.

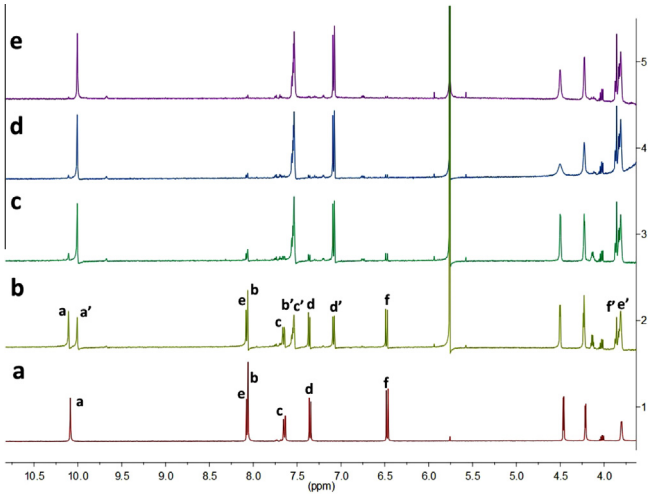

Figure 1. Partial ^{1}H NMR spectrum (500 MHz, DMSO, 25 °C) of **DAC** solution irradiated with 365 nm UV light, (a) 0 min, (b) 30 min, (c) 60 min, (d) 80 min, (e) 100 min.

The photo-induced conversion can also be convinced by the mass spectrum. Before irradiation, a peak at m/z 571.9 belonging to $[M+Cl]^-$ is found in Figure S4a. After irradiation for 100 min, the peak of **DAC** monomer disappears with only the presence of $[2M+Cl]^-$ at m/z 1107.5 (Fig. 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 spectra 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–π^* transition related to the carbonyl function, was decreased gradually and finally disappeared when irradiated for 100 min, indicating the occurrence of coumarin photo-dimerization (Fig. 2A).[11] Figure S5 displayed very rapid reaction kinetics, where the 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 ^{1}H NMR spectrum, while at higher energies there is a strong π–π^* transition band at 260 nm corresponding to the conjugated π system, which showed a continuous intensity decrease.[5h] The absorption spectrum changes may be due to the length of the conjugated π system being 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 indicates the photodimerization of coumarin groups (Fig. 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 (Figs. S6 and 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 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_{DMSO}: $V_{H2O} = 2{:}1$), 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 Figures 3A and S9 clearly revealed that the assemblies of **DAC** have

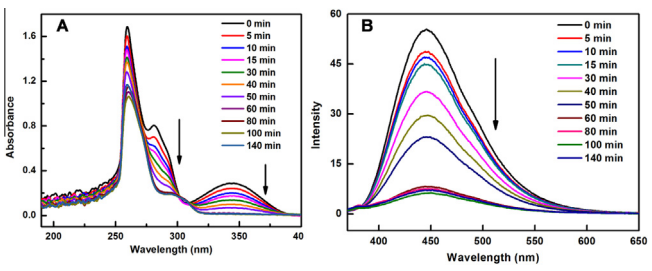

Figure 2. The absorption (A) and fluorescence (B) spectrum changes of **DAC** in DMSO solution on irradiation at 365 nm, 1×10^{-4} M, 25 °C.