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Spectral study on conformation switchable cationic calix[4]carbazole serving as curcumin container, stabilizer and sustained-delivery carrier

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ABSTRACT

A fluorescent 2,7-dimethoxy-substituted calix[4]carbazole (**1**) is facilely synthesized. The spectral behaviors of both the guest-induced switchable conformation of **1** and its abilities serving as the stabilizer and molecular carrier of curcumin are investigated. UV–vis, fluorescence and NMR spectral results show that upon binding to curcumin, the 1,3-alternate conformation of **1** is converted to be the cone one. The relative high association constant ($6.4 \times 10^6\ M^{-1}$) of **1** binding to curcumin enables it to stabilize the curcumin, to suppress its degradation, and to sustainably deliver it into the EYPC vesicles within 20 h. Moreover, the cytotoxicity assay shows that **1** does not interfere the antiproliferative activities of curcumin. All these properties endow **1** the potential capability of serving as the molecular drug carrier. Our current result may pave the way looking for more efficient fluorescent calixcarbazoles and thereof spectral utilities.

1. Introduction

One of the fascinating utilities of macrocycles is to serve as molecular drug containers [1]. Molecular containers could improve the solubility of insoluble drugs, enhance their stability, penetrate cell-membrane high effectively, thus receiving more and more interests recently [1]. There are actually quite a bit of poorly soluble and in-stable drugs so far. Take the curcumin, a naturally occurring drug isolated from the herb *Curcuma longa* [2], for an example. Although it receives much attention due to its pharmacological applications [3], the drawbacks of both its poor solubility and its instability hinder it practically therapeutic utility [4]. Lots of efforts have been made to improve the delivery systems of curcumin, but its most popular macrocyclic molecular carrier is perhaps still cyclodextrin and/or its derivatives [5].

Cyclodextrin possesses a hydrophobic cavity and could be used to encapsulate a drug and to improve its solubility and stability. Aside from cyclodextrins, some other macrocyclic molecules, such as cyclophanes, calixarenes, cucurbit[*n*]uril and self-assembled capsules, have also been developed to serve as the molecular carriers for insoluble and unstable drugs [1,6]. Each macrocyclic host has its distinguished advantage and its own limitation. As Isaacs and Briken groups [7] recently suggested that conformation-rigid macrocycles could bind strongly to the guests, whereas the "pre-organized yet sufficiently flexible" acyclic receptors bind more hydrophobic drugs, own more appropriate dissociation kinetics, and thus possessing a more promising utility as drug delivery agents.

Being different from those rigid macrocycles (e.g. cyclodextrin, cucurbit[*n*]urils, pillararenes), calixarenes belong to such a "pre-organized yet sufficiently flexible" category due to their switchable conformations. The pity is that the traditional phenol-based calix[4]arenes are too small to accommodate drug molecules. Our group recently report the syntheses of 2,7-dimethoxy-substituted calix[4]carbazole [8]. Compared with traditional calixarenes, calix[4]carbazoles possess large sizes, which could accommodate molecular guests. Moreover, they are fluorescent, so that the guest binding behaviors could be revealed by their decent chromophoric properties. Theoretically, these carbazolyl macrocycles could adopt different conformations including cone, partial cone, 1,2-alternate, 1,3-alternate, chair and boat ones and so on. These conformations could be mutually converted by external stimulus. In the previous work, however, we only observed the 1,3-alternate conformation (or bis-tweezer conformation). In order to gain insight into the versatile conformations of calixcarbazoles and thereof utility as the drug carrier, we then synthesized a novel water soluble calix[4]carbazole (compound **1**, Fig. 1). Compared with our previously investigated one [8], **1** is easier to be made. And moreover, its conformation may be less pre-organized and more flexible due to the relatively longer distance between its hydrophilic ammonium head and its hydrophobic carbazolyl moieties.

With this "pre-organized but sufficiently flexible" [7], chromophoric calix[4]carbazole at hand, we then investigated and found the spectral

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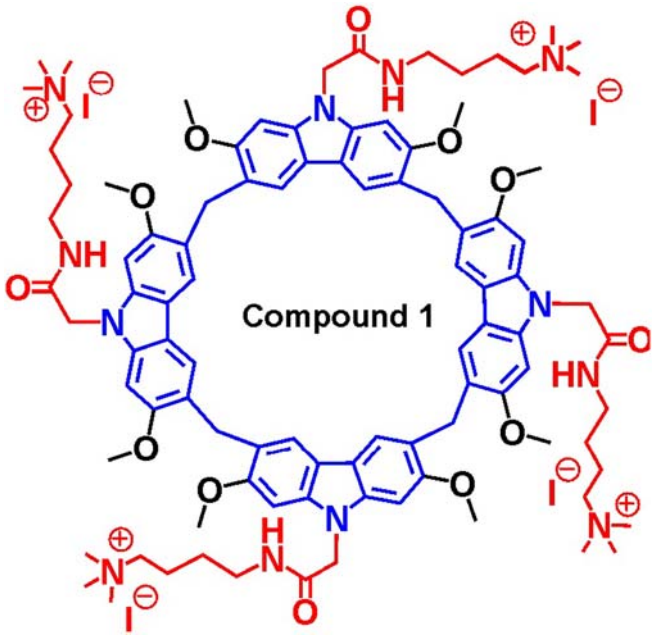

Fig. 1. The structure of compound **1**.

behavior of **1** upon binding, stabilizing as well as sustained delivering curcumin. Herein, we report our findings. And we think that our current result may shed the light on the searching of versatile conformations of calixcarbazoles and thereof spectral applications.

2. Experimental

2.1. General techniques

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Thin layer chromatography (TLC) analysis of reaction mixtures was performed on dynamic adsorbents silica gel F-254 TLC plates. Column chromatography was performed on silica gel 200–300 mesh. Fluorescence emission spectra were obtained using Shimadzu RF-5301 PC Spectrofluorophotometer. UV–vis absorption spectra were recorded on Beijing Purkinje TU-1810. Dynamic light scattering (DLS) measurements were performed on NICOM™ 380 ZLS, in all cases using quartz cuvettes (1 cm). ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded with Bruker AVANCE-III 600 spectrometers at 298 K. Chemical shifts were reported in units (ppm) and all coupling constants (J values) were reported in Hertz (Hz). High resolution mass spectra were obtained using Bruker micrOTOF-Q instrument with an ESI source.

For all the measurements, the solutions of **1** and curcumin were freshly prepared before use. For UV–vis, fluorescence and ^{1}H NMR titrations, the stock solutions of **1** and curcumin were prepared by dissolving them in DMSO (10 mM and 20 mM respectively). Both UV–vis and fluorescence measurements were performed in a 1 cm cuvette. Excitation and emission slit widths were 3 nm and 5 nm respectively. For fluorescence titration measurement, excitation wavelength was 310 nm. For curcumin delivery experiment, the excitation wavelength was 425 nm.

All the experiments were repeated at least three times.

2.2. Synthesis

The synthesis of **1** followed our previously developed methods [8] with slight modification. The detailed synthetic procedures of both the intermediates and target compound **1** and all the spectra for their characterizations had been listed in Supporting Information.

Compound 1: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.22 (t, 4H), 7.14 (s, 8H), 7.03 (s, 8H), 5.13 (s, 8H), 3.90 (s, 8H), 3.83 (s, 24H), 3.23 (t, J = 6.0 Hz, 8H), 3.16 (q, $J_1 = J_2$ = 6.0 Hz, 8H), 2.94 (s, 24H), 1.64 (m, 8H),1.45 (m,8H). ^{13}C NMR (150 MHz, DMSO d_6) δ 168.1, 156.0, 140.6, 120.7, 119.7, 115.6, 92.3, 65.2, 56.2, 52.5, 38.2, 34.8, 26.4, 19.9. HRMS (ESI/TOF-Q) Calcd. For $[M\text{-}3I]^{3+}$: 589.6279; found: 589.6298. For $[M\text{-}4I]^{4+}$: 410.4947; found: 410.4989.

2.3. Determination of fluorescence quantum yield

The fluorescence quantum yield of **1** in water was determined by a relative comparison procedure using quinine as standard (Φ = 0.54 in 0.05 M sulfuric acid): Φ = 0.043.

Before determination of the fluorescence quantum yield, the absorption of **1** was firstly measured and it was no higher than 0.05. The general equation used in the determination of relative quantum yields is as follows:

$$\Phi_u = (\Phi_s \times F_u \times A_s \times \eta_u^2)/(F_s \times A_u \times \eta_s^2),$$

where Φ is the quantum yields, F is the integrated area under the corrected emission spectrum, A is the absorption at the excitation wavelength, η is the refractive index of the solution and the subscripts 'u' and 's' refer to the unknown and the standard, respectively.

2.4. Determination of association constant

Association constants were calculated based on the data of fluorescence titration.

K_a was determined by a nonlinear least-squares analysis of $\boldsymbol{Y}$ versus $\boldsymbol{x}$ using the following equation for 1:1 stoichiometry binding [9]:

$$F_0/F = 1 + K_a[L]$$

where $[L]$ is curcumin concentration, F_0 is the fluorescence of 1 in the absence of curcumin, F is the fluorescence of **1** in the presence of curcumin, and K_a is the association constant.

2.5. Determination of curcumin stability

Following the reference [5d], the degradation of curcumin in the absence and presence of **1** were determined by UV–vis spectroscopy. In brief, the time-dependent UV–vis spectra of curcumin (10 μM) with **1** at the concentrations of 0, 10, 50, 100 μM were respectively recorded at 37 °C in 10 mM PBS buffer (pH = 7.35). The absorption at 430 nm in UV–vis spectra was plotted as a function of time. The obtained curves were fitted by using Prout–Tompkins mode l [10] to give the half-life of degradation. The correlation coefficients for all the fitted curves are higher than 0.99.

2.6. Curcumin delivery

2.6.1. EYPC membrane preparation

Egg yolk phosphatidylcholine large unilamellar vesicles (EYPC LUVs) were prepared based on the reported method [11]. The stock solution was prepared by dissolving 50 mg EYPC in 0.5 mL $CHCl_3$. 0.015 mL of the above stock solution was dropped into 20 mL $CHCl_3$, and then dried under vacuum to form thin films. The resulting thin films were hydrated with 0.1 mM HEPES buffer (pH = 7.35) for more than an hour and subsequently subjected to freeze-thaw cycles (5×) and extrusions (Mini-Extruder with a stacked polycarbonate membrane of pore size 0.1 μm). The diameter of the prepared EYPC was determined to be 133.8 nm (PDI, 0.116), as shown in Fig. S15.