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Host-guest inclusion system of ferulic acid with *p*-Sulfonatocalix[n]arenes: Preparation, characterization and antioxidant activity

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ABSTRACT

The inclusion complexes of ferulic acid (FA) with *p*-Sulfonatocalix[n]arenes (SCXn, n = 4, 6, 8) were prepared and characterized both in the solid state and in solution using fluorescence spectroscopy, ^{1}H nuclear magnetic resonance (^{1}H NMR), attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR), atomic force microscopy (AFM) and differential scanning calorimetry (DSC). The results show that FA is able to form inclusion complexes with SCXn in a molar ratio of 1:1, causing a significant decrease in the fluorescence intensity of FA. The association constant of the inclusion complexes was calculated from the fluorescence titration data. ^{1}H NMR spectroscopy analysis demonstrates that the aromatic ring and methoxy group of FA are partially covered by SCXn.

1. Introduction

Ferulic acid [2E-3-(4-hydroxy-3-methoxyphenyl)-prop-2-enoic acid] (FA) a major active constituent in many natural Chinese medicinal herbs such as Angelica sinensis, Cimicifuga racemosa and Ligusticum chuangxiong, is a type of hydroxycinnamic acid that can also be found in many fruits and vegetables [1,2]. FA exhibits a variety of biomedical, pharmaceutical and industrial applications due to its wide variety of biological activities such as anti-inflammatory, antimicrobial, anti-allergic, antithrombotic, anti-cancer and vasodilatory actions [3–5]. However, the low oxidative stability, poor thermal and photostability of FA severely restrict its wide application in medicinal and food formulations [6–8]. These problems could be resolved by inclusion complex formation with host compounds such as cyclodextrins and calixarenes.

Calixarenes are macrocyclic molecules made up of phenol linked by methylene groups and have been considered as the third generation supramolecular host molecules after crown ethers and cyclodextrins [9]. Calixarenes have been of potential interest in host-guest chemistry because they can form stable host-guest complexes with various organic, inorganic, and biological guest molecules, showing high molecular selectivity and the engaging supramolecular recognition ability [10–12]. However, most calixarenes have some limitations in practical applications due to their poor water-solubility [10,13,14]. As a fascinating family of water-soluble calixarene derivatives, *p*-Sulfonatocalix[n]arenes (SCXn, n = 4, 6, 8) have gained considerable attention during the last decades [15–18]. Possessing flexible, three-dimensional and π-electron rich cavity and hydrophilic heads (the anionic sulfonate groups), SCXn are particularly beneficial building elements for the creation of diverse nanostructures [18,19]. Moreover, SCXn are highly water-soluble and biocompatible, which make them potentially useful for diverse range of biological and pharmaceutical applications [17,20–23]. Exhibiting very low toxicity, which is an important prerequisite for novel therapeutic agents [24], SCXn have been widely studied as new platforms for increasing drug solubility and efficacy [24,25].

Various techniques such as UV-vis [26], fluorescence [27], circular dichroism [28], FTIR and NMR spectroscopy, equilibrium dialysis [29] and potentiometry [30,31] have been used for studying the supramolecular complex formation. In this study, the

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complexation of SCXn with FA were investigated by several analytical techniques including UV-Vis, fluorescence, ATR-FTIR, NMR, DSC and AFM. The stoichiometry and the association constant of the complexes were calculated. The experimental results indicated that both the stability and the antioxidant activity of FA were increased by the formation of inclusion complex with SCXn, which may extend the application of FA in the food and pharmaceutical industries.

2. Experimental

2.1. Materials and apparatus

FA, as shown in Fig. 1a, was purchased from Junsei Chemical Co., Ltd. (China). *p* -Sulfocalix[n]arenes (SCXn, n = 4, 6, 8, Fig. 1b) were provided by the Third Reagent Factory of Shanghai (China) and used as received. The stock solutions of SCXn (1×10^{-2} M) and FA (1×10^{-4} M) were prepared in double-distilled water. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical (95%) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Phosphate buffer solutions were used to adjust pH. Unless otherwise stated, all chemicals and solvents were of analytical grade. Doubly distilled water was used throughout.

2.2. Preparation of the inclusion complex of FA and SCXn

The complexes were prepared according to the previous method [32]. Inclusion complexes (SCX4/FA, SCX6/FA and SCX8/FA) were obtained by mixing aqueous solutions of SCXn and FA in a host to guest molar ratio of 1:1. The resulting solution was stirred for 48 h at room temperature to reach the equilibrium. The solution was frozen-dried using a Labconco freezee dry system(Labconco Freezone 4.5) and stored at 253 K until further use [33].

2.3. Fluorescence spectroscopy

Fluorescence spectra were recorded using a Hitachi F-2500 FL spectrofluorometer (Hitachi, Tokyo, Japan). 1.0 mL of 1×10^{-4} M FA stock solution was added into a 10.0 mL comparison tube, and then different volumes of 1×10^{-2} M of SCXn stock solutions were added. Spectral data were recorded after each addition (E_X = 325 nm, slit 10/10 nm).

2.4. Attenuated total reflection - fourier-transform infrared (ATR-FTIR) spectroscopy

ATR-FT-IR absorption spectra were recorded on an infrared spectrometer (Thermo Scientific Nicolet iS50) equipped with a sampling accessory diamond window. The spectra were collected from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} using 16 scans in order to exploit the instrumental built-up noise reduction algorithm. The spectra were collected in transmittance mode.

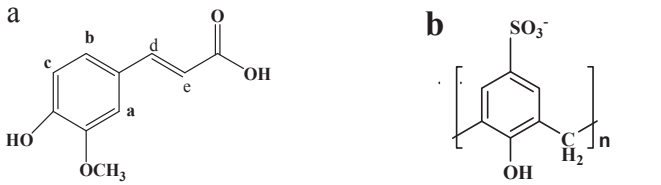

Fig. 1. Chemical structure of FA (a) and p -Sulfocalix[n]arenes (SCXn, n = 4, 6, 8) (b).

2.5. Atomic force microscopy (AFM)

Atomic force microscopy (AFM) was obtained using a multimode 8 Atomic force microscope (Bruker, Germany) in tapping mode in air. Samples for AFM imaging were prepared by dropping an aqueous solution on mica.

2.6. Nuclear magnetic resonance (NMR) spectroscopy

^{1}H NMR spectra were recorded on a Bruker DRX300 spectrometer (Fällenden, Switzerland) in D_2O using tetramethylsilane (TMS) as an internal standard.

2.7. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analysis was performed with a DSC-60 thermal analyzer (Shimadzu, Japan). The samples sealed in the aluminum crimp cell were heated at 10 °C/min from 30 °C to 500 °C in a nitrogen atmosphere.

2.8. Assay of antioxidant activities

The antioxidant activities of FA/SCXn inclusion complexes were assessed and compared with that of free FA using a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [34]. Ethanol/water (9:1, v/v) medium was used as a reference solution. Briefly, 0.5 mL sample solution was mixed with 3.0 mL of 60.0 μM DPPH in methanol. The mixture was shaken vigorously and left to stand for 30 min in the dark, and then the absorbance was measured at 517 nm against a solvent blank. The scavenging activity on DPPH radicals was calculated according to the following equation:

$$AU\% = [1 - (Ai - Aj)/Ac] \times 100\% \quad (1)$$

where AU% represented the radical-scavenging activity, Ac was the absorbance of the control solution (0.5 mL reference solution in 3.0 mL of DPPH solution), Ai was the absorbance in the presence of sample solution in DPPH solution and Aj was the absorbance of the reference solution without DPPH, which was used for error correction arising from unequal color of the sample solutions.

2.9. Stability experiments

The thermal stability was investigated at pH 6.50. The solution was kept at 80 °C for 2 h, and the absorption intensity was subsequently recorded every 30 min. Meanwhile, the photostability of the system was also studied. The solution was irradiated under an ultraviolet lamp for 2 h and the absorbance was also recorded at set time intervals.

3. Results and discussion

3.1. Fluorescence analysis

As shown in Fig. 2, FA exhibits strong fluorescence at 450 nm, which could be assigned to intraligand π-π* transition. When different volumes of SCX [4] solution was added, however, the fluorescence intensity of FA was remarkably decreased, indicating that SCX [4] formed inclusion complexes with FA. Similar results were obtained for SCX6 (Fig. S1) and SCX8 (Fig. S2). The results showed that static quenching occurred together with the complex formation. SCXn have three-dimensional and π-electron-rich cavities with multiple sulfonate groups, which could include FA into their hydrophobic cavities with the help of strong electrostatic and π-π* interaction to form inclusion complexes and may affect the