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Novel water-soluble fisetin/cyclodextrins inclusion complexes: Preparation, characterization, molecular docking and bioavailability

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ABSTRACT

Novel water-soluble inclusion complexes for fisetin (FIT) were developed by introducing β-cyclodextrin (β-CD) and γ-CD. Properties of the obtained complexes, as well as the interactions between each component, were systematically investigated in both solution and solid states by means of ESI-MS, NMR, FT-IR, XRD, DSC, SEM etc. All characterization information demonstrated that FIT/CDs inclusion complexes were formed, and exhibited different spectroscopic features and properties from FIT. A complex with 1:1 stoichiometry of FIT and CDs was confirmed with Job's method. Meanwhile, as supported by molecular modeling calculations, we suggested that phenyl group (C ring) of FIT molecule was included in the CDs cavity from the wide side. Moreover, the water solubility of FIT/CDs was successfully improved from 2.8 mg/mL (in ethanol aqueous solution) to 4.5 mg/mL (FIT/β-CD complex) and 7.8 mg/mL (FIT/γ-CD complex), and higher thermal stability results were shown by thermal analysis for those complexes. Notably, the inclusion complexes displayed almost two times higher cytotoxicity compared to free FIT against Hela and MCF-7 cells. These results suggested that FIT/CDs complexes could be potentially useful in food industry and healthcare area.

1. Introduction

Flavonoids are a group of low molecular weight polyphenolic compounds widely distributed in plants exhibiting a variety of biological activities including anti-oxidant, anti-inflammatory and anti-tumor effects.[1,2] Fisetin (FIT), also namely 3,3′,4′,7-tetrahydroxyflavone(5-deoxyquercetin), is a naturally occurring flavonoid abundantly found in fruits, vegetables and anacardiaceae plants (*Rhussuccedanea L*)[3] such as strawberries, grapes, onions. Recently, pharmacological studies and clinical practice have demonstrated that FIT fulfilled a broad spectrum of biological functions, including anticancer,[4] antiangiogenic,[5] neuroprotective,[6] neurotrophic,[7] anti-inflammatory,[8] and antiproliferative.[9] Therefore, it is considerably interesting to introduce FIT into medicines as well as nutritional supplements.[10] However, similar to many natural herbal medicinal components, the usage of FIT is greatly limited by its low water solubility (<1 mg/mL)[11] and low bioavailability.

Considering the important usage of FIT, developing a practical water-soluble method has become increasingly important for clinical applications. To enhance the water solubility of the biologically active components, various formulations such as the formation of amorphous solids, nanoparticles, micro-emulsions, solid dispersions, water-soluble complexes, as well as the techniques of melt extrusion, salt formation are investigated.[12,13] Among these methods, formation of inclusion complexes with other water-soluble components, which could provide carriers for the aim component, is an attractive and effective technique. In addition, increased stability, dissolution rate and bioavailability have also been observed by forming inclusion complexes.[14–16]

Cyclodextrins (CDs) are a family of molecules composed of five or more glucopyranoses bonding together in a ring. Alpha-, beta- and gamma-CDs consisting of six, seven, and eight α-D-glucopyranose units respectively, have been intensively studied. In this regard, β- and γ-CDs are torus-shaped oligosaccharides containing seven or eight glucose units (Fig. 1B) with a hydrophilic outer surface and a lipophilic inner cavity that can accommodate a wide variety of lipophilic drugs, forming the so-called inclusion complexes. More recently, our group reported that the water solubility

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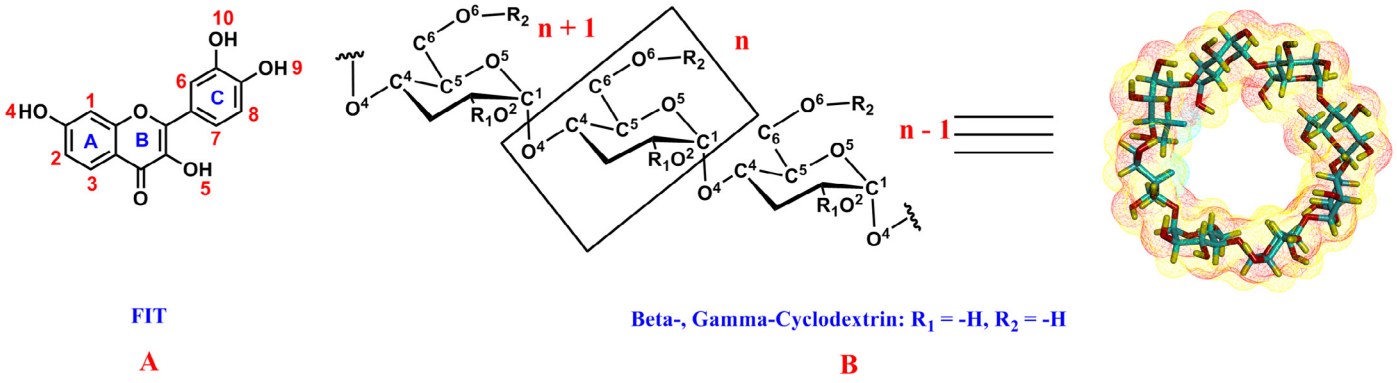

Fig. 1. Chemical structure of FIT (A) and β-, γ-CD (B).

and bioavailability of natural products were significantly enhanced by generating inclusion complexes with CDs.[17–20]

In this work, inclusion complexes of FIT have been developed for the first time in combination with β- and γ-CDs to increase the aqueous solubility of FIT. To enhance the practicability, a simple freeze-drying method has been employed. UV–*vis* spectroscopy was measured to evaluate the FIT/CDs complexes in aqueous solutions. In order to verify the formation of the inclusion complexes, drug/CD interactions in the solution were investigated using a molecular modeling analysis, and interactions in the solid state were characterized using NMR, FT-IR, XRD, SEM, TG and DSC analyses. The binding stoichiometry of the complexes was confirmed with Job's method and ESI-MS[21] examinations. Finally, the inhibitory effects of FIT/CDs on cell growth were observed with enhanced activity compared with free FIT.

2. Materials and methods

2.1. Materials

FIT ($C_{15}H_{10}O_6$, FW = 286.23, purity >99%) was obtained from Sigma-Aldrich (Dorset, UK). β-CD (FW = 1135, purity >99%) and γ-CD (FW = 1297, purity >99%) were purchased from *Adamas Reagent Co. Ltd.* and used without further purification. Other reagents and chemicals were of analytical reagent grade. All experiments were carried out using ultrapure water.

2.2. Preparation of the inclusion complexes and physical mixtures

The FIT/CD inclusion complex in 1:1 ratio was prepared using the freeze-drying method.[22] β- and γ-CD in accurate weights were dissolved in ultrapure water. Subsequently, a solution of FIT in ethanol was slowly added into the pre-prepared aqueous CD solution. The resulting suspension was stirred at room temperature for 48 h. It was subsequently filtered through a 0.22-μm membrane filter before being lyophilized with a freeze dryer (Alpha2-4, Christ) to afford the solid complexes. The physical mixtures employed as comparative trials were prepared by mixing the powders in a 1:1 molar ratio of FIT and CDs with an agate mortar.

2.3. Stoichiometry determination: Job's method and phase-solubility studies

The UV–*vis* absorption spectra of FIT, CDs and the inclusion complexes were recorded using a UV-2401 spectrophotometer (Shimadzu, Tokyo, Japan) in the range of 200–600 nm. The stoichiometry of the inclusion complex was determined using the continuous variation Job's method.[23,24] During the systematical examination, solutions of FIT (7.6×10^{-5} M) and CDs (7.6×10^{-5} M) were mixed to a standard volume containing a fixed total concentration of the species. In the solutions, the R (R = [FIT]/([FIT] + [CDs]), [FIT] + [CDs] = 1.52×10^{-4} M) is systematically varied from large to small. The maximum amount of the complex FIT should occur at the stoichiometric ratio from 0.0 to 1.0.

An excess amount of FIT was added to CD solutions of different concentrations (2–20 mM). The resulting suspensions were sonicated for 10 min and shaken (100 rpm) for 24 h in ethanol aqueous solution (V_{water}:$V_{ethanol}$ = 4:1) at 25 °C until a solubility equilibrium was established. Next, the suspensions were filtered through Millipore membrane filters (0.45 μm pore size). Then, a UV spectrophotometer at 358 nm was used to detect the obtained solutions using a UV–*vis* spectrophotometer (Shimadzu UV 2401, Japan). The binding constant (K_a) was calculated from a phase-solubility diagram with the assumption of 1:1 stoichiometry using the following Higuchi and Connors[25] equation:

$$K_a = \text{Slope}\left[\text{Intercept}(1-\text{Slope})^{-1}\right] \quad (1)$$

2.4. Characterization of inclusion complexes[26]

2.4.1. NMR spectroscopy

^{1}H NMR and ROESY spectra were obtained on an Ascend 500 MHz NMR spectrometer (Bruker, Switzerland) using D_2O or DMSO-d_6 as a solvent. Chemical shifts were reported in ppm with tetramethylsilane (TMS) as an internal standard.

2.4.2. Fourier-transform infrared spectroscopy (FT-IR)

FT-IR spectra were recorded on the Fourier transform infrared spectrometer (Nicolet 6700, Thermo Fisher Scientific, Waltham, MA) according to the KBr disk technique. Samples were prepared as KBr disks with 1 mg complex and 100 mg KBr. The FTIR measurements were performed in the scanning range of 4000–400 cm^{-1} at ambient temperature.

2.4.3. Powder X-ray diffractometry (XRD)

X-ray powder diffraction patterns were performed on a Philips X'Pert Pro diffractometer using Ni-filtered and Cu Kα radiation (45 kV, 35 mA). The scanning rate employed was 0.15°/min in a diffraction angle (2θ) range of 3°–50°.

2.4.4. Scanning electron microscopy (SEM)

The surface morphology of the drug, physical mixture, and inclusion complex were examined by scanning electron microscope (SEM). The samples were fixed on a brass stub using double-sided tape and made electrically conductive by coating with a thin layer