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Experimental and molecular docking investigations on the inclusion mechanism of the complex of phloridzin and hydroxypropyl-β-cyclodextrin

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ABSTRACT

Phloridzin is a nutraceutical. Its use in food, medicine and cosmetics is limited because of its low aqueous solubility and stability limits, but it can be improved by complexing with cyclodextrins. In this study, we investigated the inclusion mechanism between phloridzin and hydroxylpropyl-β-cyclodextrin (HP-β-CD) using isothermal titration calorimetry (ITC), ultraviolet–visible spectrometry (UV), infrared spectrometry (IR), proton nuclear magnetic resonance spectroscopy (^{1}H NMR) and molecular docking simulations. The ITC results found that the equilibrium binding constant of HP-β-CD with phloridzin was higher than that of β-CD. Their inclusion was a spontaneous process with negative ΔG, ΔH and ΔS values. UV spectra showed that the aqueous solubility of phloridzin was enhanced by HP-β-CD. Our IR analysis verified the inclusion complexation of phloridzin into the HP-β-CD cavity. The Autodock determined that the substitution distribution of HP-β-CD influenced not only the orientation and depth degree of phloridzin within the cavity, but also the binding energies.

1. Introduction

Phloridzin is a dihydrochalcone primarily found in apples and *Lithocarpus polystachyus Rehd* (a traditional Chinese sweet tea) with many health-promoting functions, such as antioxidant, antidiabetic, antitumor and anti-inflammatory activities (Dong et al., 2007; Gosch, Halbwirth, & Stich, 2010; Najafian et al., 2012). Due to its poor aqueous solubility and stability, its application in the food, medicine and cosmetic industries is limited, but it can be improved by complexing with cyclodextrins (CDs) (Pinho, Grootveld, Soares, & Henriques, 2014). The inclusion of phloridzin into β-CD was previously studied as a model of molecular recognition in membranes by using NMR spectra and the DADAS90 program (Ishizuka et al., 2002). β-CD is the most useful CD, but the strong intramolecular hydrogen bonds of β-CD lead to its poor solubility in water (1.85 g/100 mL at 25 °C) (Liu et al., 2015). Hydroxylpropyl-β-cyclodextrin (HP-β-CD) is the hydroxyalkyl derivative of β-CD with a higher aqueous solubility (>500 g/L, 20 °C), and was approved for use in foods and medicines by the F.D.A. (Szente & Szejtli, 1999; Yuan, Jin, & Li, 2008). Prior studies investigated their complexation with phenolic compounds (Jullian, Moyano, Yãnez, & Olea-Azar, 2007; Liu, Qiu, Gao, & Jin, 2006; Yuan, Liu, Li, and Li, 2012), and characterized the phloridzin/HP-β-CD complex in its solid state. However, these studies did not determine the inclusion mechanism and three-dimensional supermolecular structure. Since the hydroxylpropyl groups spread randomly among the different glucose units of HP-β-CD, it was difficult to determine the effect of the distribution of 2-hydroxypropyl groups of HP-β-CD on its complex ability. In our study, we investigated the inclusion mechanism between phloridzin and HP-β-CD using isothermal titration calorimetry (ITC), ultraviolet spectrometry (UV), infrared spectrometry (IR) and proton nuclear magnetic resonance spectrometry (^{1}H NMR). We also studied the distribution of hydroxylpropyl groups on the inclusion process and the supermolecular structures of phloridzin/HP-β-CD complex using a molecular docking simulation, which could prompt the application of phloridzin in food industry and guide the molecular design of HP-β-CD.

2. Materials and methods

2.1. Chemicals

Phloridzin (purity 98.0%) was from purchased from Aladdin (Shanghai, China). (2-hydroxypropyl)-β-cyclodextrin (average

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MW 1380) and β-CD (MW 1134.98) were purchased from Sigma (St. Louis, MO, U.S.A.). Ultrapure water was obtained from a GenPure UV/UF laboratory ultra pure water system (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) and was used throughout our experiments.

2.2. Isothermal titration calorimetry (ITC)

ITC measurements were performed on a Nano ITC Standard Volume instrument (TA Instruments-Waters LLC., New Castle, DE, U.S.A.) according to the previous method (Zheng et al., 2014). The titrant was the aqueous solution of CD or HP-β-CD (12 mM), which was degassed before each test. The aqueous solution of phloridzin (1.2 mL, 0.2 Mm) was loaded into the sample cell with 1.2 mL of ultrapure water in the reference cell as a blank. Then, 33 portions of the 3 μL titrant in a 100 μL ITC stirrer syringe were injected into the sample cell at 25 °C with enough sufficient interval times, which were controlled by ITCRun software. The titration of the CD solution into water conducted at the same conditions to eliminate the thermal effect due to dilution. All experiments were repeated in triplicate. The data was processed by the TA Nanoanalyze 3.3 software.

2.3. Ultraviolet–visible spectroscopy (UV)

Excess amounts of phloridzin (50 mg) were mixed with 5 mL of aqueous solution of HP-β-CD ranging from 0 to 10 mmol/L. Then the mixtures were shaken in the water bath at 25 °C for 72 h and filtered. The supernatant (50 μL) was diluted to 10 mL with ultrapure water for UV analysis. The UV spectra were determined using a UV–visible spectrophotometer with the scanning range from 220 to 400 nm (Kim, Kim, & Jung, 2008).

2.4. Fourier transform infrared spectroscopy (FT-IR)

We prepared the complex and physical mixture of phloridzin and HP-β-CD for the FT-IR analysis. Phloridzin (0.118 g, 0.25 mM) and HP-β-CD (0.345 g, 0.25 mM) were mixed in 40 mL of ultrapure water, stirred for 24 h at 30 °C and then filtered. The supernatant was lyophilized and collected as the complex. Phloridzin and HP-β-CD at the above ratio were uniformly mixed in the solid state at 30 °C and collected as their physical mixture. A Bruker Tensor 27 FT-IR spectrophotometer was used to obtain the IR spectra in the range from 4000 to 400 cm^{-1} by using the KBr method (Eid et al., 2011).

2.5. Proton nuclear magnetic resonance spectroscopy (^1H NMR)

The ^1H NMR measurements were carried out by using a Bruker 600 MHz AVANCE spectrometer at 25 °C. Phloridzin (30 mg) and HP-β-CD (100 mg) were mixed with 5 mL of D_2O, shaken at 30 °C for 72 h and filtered. The supernatant was collected as the phloridzin/HP-β-CD complex for ^1H NMR. The ^1H NMR spectra of phloridzin in D_2O were measured at the same conditions. The chemical shifts (δ) were reported as ppm and referenced to the HOD signal (Liu et al., 2015).

2.6. Molecular docking simulation

To clarify the distribution of the hydroxypropyl group of HP-β-CD on its interaction with phloridzin, five structures of HP-β-CD were prepared by adding four hydroxypropyl groups to the rims of β-cyclodextrin and were optimized by the molecular mechanics method (MM+). The binding energies and supermolecular structures of these complexes of phloridzin and HP-β-CD were determined by the Autodock 4.2 molecular docking software (The Scripps Research Institute, La Jolla, CA, U.S.A.). The genetic algorithm method was used with the initial population for the global search of 50 individuals (Morris et al., 2009).

2.7. Statistical analysis

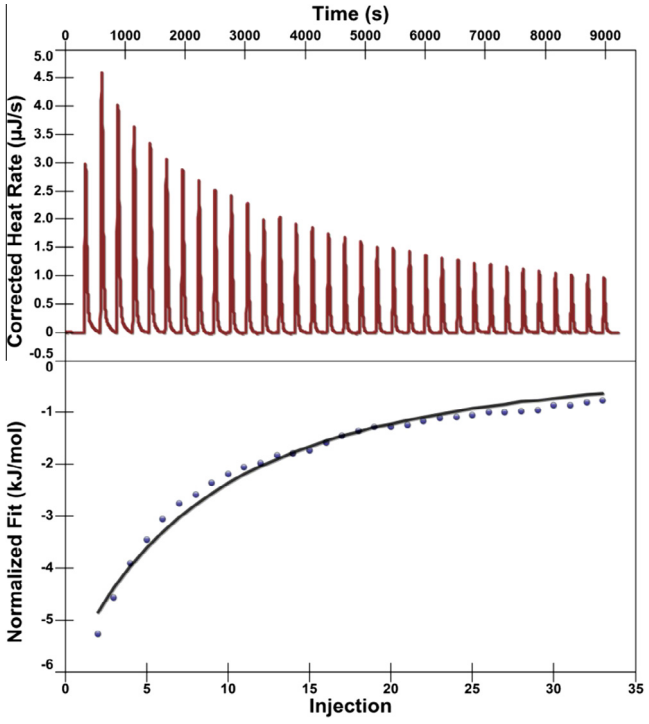
The results were expressed as the mean ± SD. The significant difference was determined by Fisher's Least Significant Difference Test (LSD).

3. Results and discussion

3.1. ITC analysis

ITC is a method to determine the equilibrium binding constants and thermodynamic parameters of the host-guest interactions, and is based on the direct measurement of heat changes during the interaction (Frazier et al., 2010; Zheng et al., 2014). In this study, the inclusion behavior of β-CD and HP-β-CD with phloridzin was compared using the ITC method. Table 1 shows the typical ITC result of the complex of HP-β-CD and phloridzin. It could be found that their inclusion was an exothermic process. When the injection time increased, the heat change became stable. The first exothermic peak for the first injection was lower than that of the second, which was a common phenomenon of ITC from dilution effect. And the data of the first injection was always excluded from the model fitting. Then, an independent model was used to fit the obtained

Table 1
Equilibrium binding constants and thermodynamic parameters of inclusion complexation of phloridzin with β-CD and HP-β-CD obtained from ITC data.



	β-CD	HP-β-CD
Ka (M^{-1})	1485 ± 30.81[a]	2091 ± 53.23[b]
ΔH (kJ/mol)	−99.99 ± 0.01[a]	−99.99 ± 0.01[a]
ΔS (J/mol·K)	−274.63 ± 0.15[a]	−271.80 ± 0.17[b]
ΔG (kJ/mol)	−23.65 ± 0.05[a]	−24.43 ± 0.06[b]

Values in the row with different letters indicate significantly difference ($p < 0.05$).