Food Chemistry 120 (2010) 837–841

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Preparation, characterisation and activity of the inclusion complex of paeonol with β-cyclodextrin

Yuhsin Tsai [a,*,1], Hsieh-Ho Tsai [b,1], Chien-Pang Wu [b], Fuu-Jen Tsai [c,d,e]

[a] School of Chinese Medicine, China Medical University, Taichung, Taiwan
[b] Nano-Powder and Thin Film Technology Center, Industrial Technology Research Institute, Tainan, Taiwan
[c] School of Post-Baccalaureate Chinese Medicine, China Medical University, Taichung, Taiwan
[d] Department of Medical Genetics, Pediatrics and Medical Research, China Medical University Hospital, Taichung, Taiwan
[e] Department of Biotechnology and Bioinformatics, Asia University, Taichung, Taiwan

ARTICLE INFO

*Article history:*
Received 24 March 2009
Received in revised form 17 September 2009
Accepted 10 November 2009

*Keywords:*
Paeonol
β-Cyclodextrin
ROESY
Antioxidant activity
Tyrosinase inhibition

ABSTRACT

The inclusion complex of β-cyclodextrin (β-CD) and paeonol (2′-hydroxy-4′-methoxyacetophenone, PAE) was synthesised and characterised by thermal gravimetric analysis (TGA) and two-dimensional rotating frame spectroscopy (2D ROESY). The antioxidant activity and tyrosinase inhibition activity were also studied. The TGA results indicated that the thermal stability of PAE was improved when it was included with β-CD. Based on the 2D ROESY analysis, an inclusion structure of the PAE–β-CD complex was proposed, in which PAE penetrated β-CD in a tilted manner due to the interaction of intermolecular hydrogen bonds between PAE and β-CD. The complex of PAE with β-CD increased the antioxidant activity and tyrosinase-inhibiting activity of PAE.



## 1. Introduction

Cyclodextrins (CDs), cyclic oligosaccharides whose molecules have hydrophilic outer surfaces and a hydrophobic cavity at the centre, have been extensively investigated because they act as host molecules or containers in the formation of inclusion compounds with many guest molecules in aqueous solution (Brewster & Loftsson, 2007; Vyas, Saraf, & Saraf, 2008; Yuan, Jin, Xu, Zhuang, & Shen, 2008). Thus, CD can increase the aqueous solubility, chemical reactivity and spectral properties of numerous lipophilic drugs which are used as guest molecules, without changing their intrinsic ability to permeate lipophilic membranes (Del Valle, 2004). Of these host molecules, β-cyclodextrin (β-CD), as presented in Fig. 1a, containing seven glucose units, is widely used to enhance the solubility, stability and bioavailability of drugs (Uekama, Hirayama, & Irie, 1998; Kang, Kumar, Yang, Chowdhury, & Hohl, 2002) and is the preferred agent for encapsulation of drugs in the pharmaceutical industry, because of its low price and high rate of production (Li et al., 2005).

Paeonol (2′-hydroxy-4′-methoxyacetophenone, PAE), as shown in Fig. 1b, is the main active compound of the *Paeonia lactiflora* Pallas, a traditional Chinese herb that is used in Asia and Europe. PAE can improve blood flow, down-regulate transcription factors NF-κB and AP-1 (Ishiguro et al., 2006; Nizamutdinova et al., 2007), suppress the expression of cyclooxygenase-2, nitric oxide synthase, cell surface adhesion molecules, TNF-α and IL-1β (Chou, 2003; Nizamutdinova et al., 2007), and inhibit the activity of extracellular signal-regulated kinase and p38 (Nizamutdinova et al., 2007; Tsai et al., 2008). This compound has antioxidant and anti-inflammatory activities and suppresses tumor formation (Chou, 2003; Chung, 1999; Nizamutdinova et al., 2007; Xie, Chen, & Ma, 2007). PAE can also inhibit melanin synthesis and down-regulate melanin transfer (Xie et al., 2007), effects which can be exploited in cosmetic applications. Although PAE has many characteristics that make it suitable for potential medical uses, it is lipophilic and has a low aqueous solubility, which may limit its range of applications. Therefore, the formation of an inclusion complex with β-CD to increase its solubility is of interest.

To the best of our knowledge, few studies have investigated the inclusion complex of PAE with CD, although the inclusion complex has been identified by UV–visible absorption spectrometry (Li & Ren, 2004) and the complexation of β-CD with PAE and its isomers has been examined using isothermal titration calorimetry and nuclear magnetic resonance (Sun, Li, Qiu, Liu, & Yin, 2006). The aims of this investigation are to study the effects of complexation on thermal stability, antioxidant activity and tyrosinase inhibition

* Corresponding author. Tel.: +886 4 22053366x1019; fax: +886 4 22032295.
*E-mail address:* yhtsai@mail.cmu.edu.tw (Y. Tsai).
[1] They contributed equally to this work.

0308-8146/$ - see front matter Crown Copyright © 2009 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.11.024

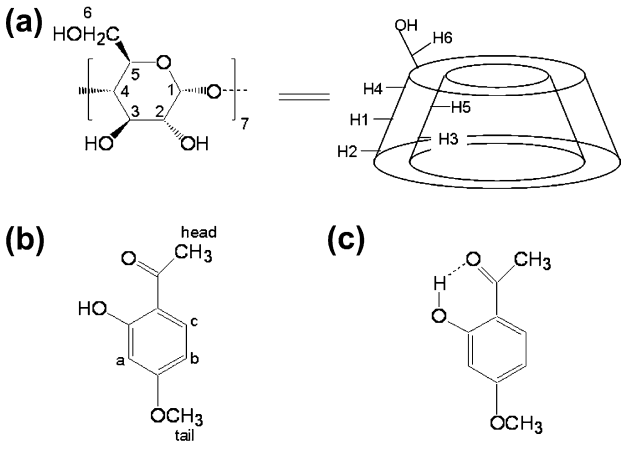


**Fig. 1.** (a) β-CD; (b) PAE; (c) PAE with intra-molecular hydrogen bond.

of PAE. Additionally, 2D ROESY was adopted to correlate these properties with the structure of the complex and a model is proposed to explain the experimental results obtained in this study.

## 2. Materials and methods

### 2.1. Materials

PAE (FW: 166), β-CD (FW: 1135), mushroom tyrosinase, L-tyrosine, DPPH (2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl) free radical, dibasic sodium phosphate ($Na_2HPO_4$) and monobasic potassium phosphate ($KH_2PO_4$) were obtained from Aldrich (St. Louis, MO, USA). All of these chemicals were of analytical grade. Ethanol (95%, v/v) was purchased from Merck Co. (Santa Ana, CA, USA). Sorensen's buffer was prepared by adding 3.4 g of $KH_2PO_4$ and 3.55 g of $Na_2HPO_4$ to 1000 ml of water. The water was doubly-distilled and deionised.

### 2.2. Preparation of PAE–β-CD complexes and physical mixtures

PAE–β-CD complex was prepared by coprecipitation. β-CD (3.5 g) was dissolved in distilled water (43.75 g) at 70 °C in an oil bath for 1 h. PAE (0.5 g) in ethanol (3.46 g) was slowly added to the β-CD solution with continuous agitation. The molar ratio of PAE to β-CD was 1:1. The vessel was sealed and stirred continuously for 6 h, 4 ml of ethanol were added dropwise to regulate the solubility of the hydrophobic solute in β-CD solution. The final solution was refrigerated overnight at 4 °C. The precipitated PAE–β-CD complex was recovered by filtration and washed with ethanol to remove unencapsulated PAE. This residue was dried in a vacuum oven at room temperature for 48 h to prevent the sublimation of PAE from the inclusion complex. The final powder was stored at 4 ° in an airtight bottle.

A physical mixture of β-CD and PAE in the same molar ratio as the PAE–β-CD inclusion complex was prepared using a mortar and pestle for 2 min to obtain a homogeneous physical mixture.

### 2.3. Thermogravimetric analysis (TGA)

TGA was performed using a thermogravimetric analyser (TA Q5000). After the sample (about 10 mg) had been loaded onto the platinum pan of the thermogravimetric analyser, analysis was carried out at a heating rate of 20 °/min in an atmosphere of nitrogen.

### 2.4. 2D ROESY

2D ROESY was recorded on a Bruker-600 MHz instrument. The samples were prepared by dissolving 3 mg of the inclusion complex in about 1 ml of $D_2O$ at room temperature. The spin-lock mixing time was set to 500 ms after calibration to ensure the reliability of ROESY cross-peaks.

### 2.5. Antioxidant activity of PAE–β-CD inclusion complex and pure PAE

The DPPH free radical-scavenging test was conducted as described elsewhere (Strazisar, Andrensek, & Smidovnik, 2008). 10 mM and 6 mM solutions of PAE–β-CD inclusion complex (or PAE) were prepared by dissolving 1.3 g and 0.78 g of PAE–β-CD inclusion complex (or 0.166 g and 0.1 g of PAE) in 100 ml of a mixed solvent (water:ethanol = 50:50, v/v), respectively. 1.5 ml of these inclusion complex (or PAE) solutions were added to 2 ml of the DPPH· solution (water:ethanol = 50:50, v/v) to reach the final concentration of inclusion complex (or PAE) of about 4.29 and 2.57 mM. The initial concentration of DPPH· solution was 0.4 mM. After incubation for 90 min at room temperature in the dark, the free radical-scavenging activity of the inclusion complex (or PAE) was measured by monitoring the decay of absorbance of DPPH· solution at 517 nm in the presence of the inclusion complex (or PAE) solution. The absorbance of the sample was compared with the absorbance of the control. The percentage scavenging activity was calculated as follows:

$$\%\ \text{scavenging activity} = 100 \times (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}$$

where $A_{\text{control}}$ was the absorbance of the DPPH· solution without the inclusion complex (or PAE) solution, and $A_{\text{sample}}$ was the absorbance of the DPPH· solution with the inclusion complex (or PAE) solution. All data were reported as means of at least three replicates.

### 2.6. Inhibition of tyrosinase

The oxidation of L-tyrosine by tyrosinase was spectrophotometrically monitored, as described elsewhere (Lim, Lim, & Yule, 2009). Mushroom tyrosinase was used without further purification. The assay was conducted in a 3.5 ml quartz cell and a series of concentrations of the inclusion complex (or PAE) in Sorensen buffer (pH 6.8) was pre-incubated with 0.3 ml of tyrosinase (230units/ml) for 10 min at 37 °C. 0.3 ml of L-tyrosine was added to initiate the reaction and the assay mixture was incubated for 20 min. The amount of dopachrome produced in the reaction mixture was measured by a UV–visible spectrometer at a fixed wavelength of 475 nm. The percentage inhibition of tyrosinase was calculated as follows:

$$\%\ \text{inhibition} = 100 \times (A_0 - A_S)/A_0$$

where $A_0$ represented the absorbance of the assay solution without the inclusion complex (or PAE) after incubation and $A_S$ represented the absorbance of the assay solution with the inclusion complex (or PAE) after incubation. All data were recorded as means of at least three replicates.

## 3. Results and discussion

### 3.1. TGA

Thermogravimetric analysis was performed at a heating rate of 20 °C/min in an atmosphere of nitrogen. Fig. 2a presents the TGA profiles of β-CD, PAE, PAE–β-CD complex and the physical mixture of PAE and β-CD. β-CD exhibited two weight loss steps at 50–100 °C and 350–375 °C. The former was associated with the