Journal of Luminescence 194 (2018) 82–90

Contents lists available at ScienceDirect

Journal of Luminescence

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

# Complex formation of flavonoids fisetin and geraldol with β-cyclodextrins

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ARTICLE INFO

*Keywords:*
Fisetin
Geraldol
Cyclodextrin
Fluorescence spectroscopy
Host-guest interaction

ABSTRACT

Fisetin (FIS) is a flavonoid aglycone which is widely distributed in several fruits and vegetables. Based on its complex biochemical action in the body, FIS seems a promising candidate for the treatment of neurodegenerative diseases, diabetes, or cancer. During the 3′-*O*-methylation of FIS by catechol-*O*-methyltransferase geraldol (GER) is formed which is also pharmacologically active. Similarly to other flavonoids, FIS and GER are poorly soluble in water. Microcapsulation of flavonoids can enhance their aqueous solubility and may improve their pharmacokinetic properties. Since both FIS and GER are fluorescent molecules, the interaction of these flavonoids by native and chemically modified β-cyclodextrins was investigated by fluorescence spectroscopy. Both FIS-CD and GER-CD complexes show considerable emission with maxima at 475 and 540 nm reflecting the two conformers of the complexes. Thereafter, fluorescence enhancement of each tautomer by cyclodextrins and binding constants of flavonoid-cyclodextrin complexes were determined. Chemically modified cyclodextrins were stronger fluorescence enhancers of flavonoids and formed more stable complexes with FIS and GER than the native β-cyclodextrin. Finally, influence of cyclodextrins on the cellular impact of FIS and GER was tested on HepG2 tumor cell line.

## 1. Introduction

Fisetin (FIS; Fig. 1) is a commonly occurring flavonoid (flavonol) aglycone in the nature. It is widely distributed in different plants including several fruits (e.g., strawberry, apple, and grape) and vegetables (e.g., onion and cucumber) [1,2]. Previous studies revealed the significant biological/pharmacological importance of this molecule because FIS can be a promising candidate for the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases due to its neuroprotective effects [3]. Recent reports suggest that FIS decreases neuronal cell death, improves learning and memory, alleviates oxidative stress, and it has anti-inflammatory activity as well [3,4]. Furthermore, previous studies also highlighted that FIS may be suitable as an anticancer agent, because it can influence several intracellular signalling pathways [5]. FIS has effect on cell survival and apoptosis, cell proliferation, cell cycle progression, microtubule assembly as well as on cell migration and invasion [6,7]. Moreover, FIS shows promising effects regarding several chronic diseases including diabetes [8,9]. Similarly to other flavonoids, FIS undergoes extensive biotransformation in the body [10]. One of the most important metabolites of FIS is its 3′-*O*-methylated derivative called geraldol (GER; Fig. 1), which is formed during the methylation of FIS by catechol-*O*-methyltransferase [11,12]. GER is an active metabolite of FIS [13,14], and based on previous studies it is reasonable to hypothesize that GER is partly responsible for the therapeutically advantageous effects of FIS [11,12]. Furthermore, GER also occurs as a flavonoid component of some plants [15,16].

FIS interacts with several molecules in the human organism; these interactions can be investigated by fluorescence spectroscopic techniques due to FIS exerts strong fluorescence under physiological conditions [17–19]. Interaction of FIS with native α-, β- and γ-cyclodextrins was also evaluated based on the fluorescence enhancement of the guest molecule by cyclodextrins (CDs) [18,20,21]. During the interaction of FIS with β-cyclodextrins, excited-state intramolecular proton-transfer and dual emission properties of FIS were observed [20]. Besides the native CDs, Banerjee and Sengupta also investigated the complex formation of FIS with succinyl-(2-hydroxypropyl)-β-cyclodextrin [20]. On the other hand, we have limited information how the chemical modifications of CDs influence the fluorescence properties of FIS and the stabilities of FIS-CD complexes. Furthermore, similar data are not available regarding the fluorescence properties of GER in the presence of CD-s or complex stabilities of GER-CD complexes. CDs are widely studied host molecules, they have a conical structure with a hydrophobic interior and a hydrophilic exterior space [22]. The internal cavity is able to accommodate

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http://dx.doi.org/10.1016/j.jlumin.2017.10.017
Received 14 June 2017; Received in revised form 18 September 2017; Accepted 6 October 2017
Available online 08 October 2017

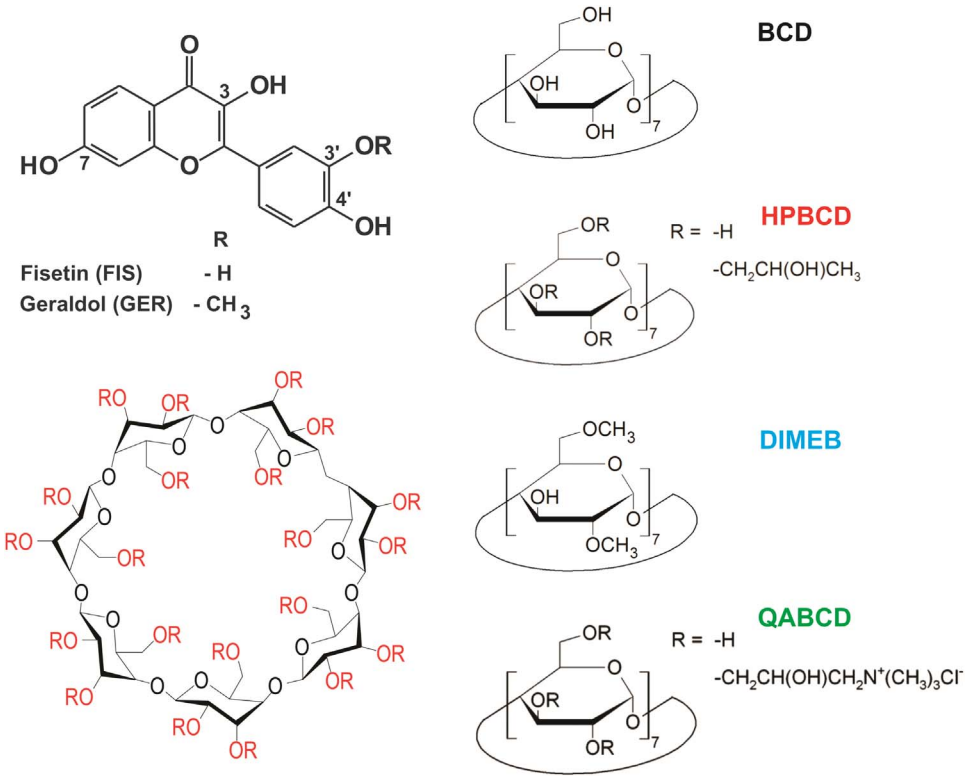


**Fig. 1.** Chemical structures of fisetin and geraldol (top left), basic structure of β-cyclodextrins (bottom left), and chemical structures of studied β-cyclodextrins (right) including native β-cyclodextrin (BCD), (2-hydroxypropyl)-β-cyclodextrin (HPBCD; randomly substituted), heptakis-2,6-di-*O*-methyl-β-cyclodextrin (DIMEB; substituted in 2 and 6 positions), and (2-hydroxy-3-N,N,N-trimethylamino)propyl-β-cyclodextrin (or quaternary ammonium β-cyclodextrin, QABCD; randomly substituted).

several guest molecules. Chemical modification of the native CDs can lead to the significant changes of selectivity and the stability of host-guest complexes [22,23]. Furthermore, parent β-CD readily forms self-assembled aggregates in water at room temperature; while chemically modified, highly water-soluble β-CDs derivatives – including hydroxypropyl-β-cyclodextrins, methyl-β-cyclodextrins, or quaternary ammonium-β-cyclodextrin – show no or low tendency for such aggregate formation. This aggregation phenomena is mainly due to the capability of BCD to self-associate via intermolecular H-bonds in water. However, statistically substituted β-CD derivatives cannot form such highly ordered H-bond systems in water, thus they form no self-assembled nano-aggregates. The cationic quaternary ammonium-β-cyclodextrin, besides having interrupted H-bond systems, due to the electrostatic repulsion will remain in aqueous solutions mainly as hydrate monomer. The fluorescence of a fluorophore guest molecules is usually modified during the complex formation, and the complexation can result in significant fluorescence enhancement [24,25]. Similarly to other flavonoids, FIS and GER are poorly soluble in water. Because CDs are able to significantly improve the solubility and pharmacokinetic behavior of drugs in several cases [26], deeper understanding of the complex formations of CDs with FIS and GER could be of high pharmacological importance.

In this study, the interaction of FIS and GER with native and chemically modified β-CDs was investigated using fluorescence spectroscopy. Because two tautomeric forms of both FIS and GER exist, the fluorescence enhancement of the tautomers as well as the stabilities of these flavonoid-CD complexes were evaluated. Since the tested chemically modified CDs showed significantly higher complex stabilities during their interactions with FIS and GER, we tested how CDs can influence the antiproliferative effects of FIS and GER on HepG2 liver tumor cell line.

## 2. Materials and methods

### 2.1. Reagents

All reagents and solvents were of spectroscopic or analytical grade. Fisetin (FIS) and geraldol (GER) were purchased from Sigma-Aldrich and from Extrasynthese, respectively. β-cyclodextrin (BCD), (2-hydroxypropyl)-β-cyclodextrin (HPBCD), heptakis-2,6-di-*O*-methyl-β-cyclodextrin (DIMEB), and (2-hydroxy-3-N,N,N-trimethylamino)propyl-β-cyclodextrin (QABCD) were from CycloLab Cyclodextrin Research & Development Laboratory, Ltd. Spectroscopic measurements were performed in phosphate buffered saline (PBS, pH 7.4) in order to mimic extracellular physiological conditions. Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich), Fetal Bovine Serum (FBS, Pan-Biotech), Bioluminescent ATP Assay Kit CLSII (Roche), Coomassie Brilliant Blue G-250 (Reanal), and bovine serum albumin (BSA, Biosera) were used as received.

### 2.2. Fluorescence spectroscopic measurements

Steady-state fluorescence measurements were performed at + 25 °C applying a Hitachi-F4500 fluorescence spectrophotometer. Stabilities of FIS-CD and GER-CD complexes were evaluated employing the graphical application of the Benesi-Hildebrand equation, assuming 1:1 stoichiometry:

$$\frac{I_0}{(I - I_0)} = \frac{1}{A} + \frac{1}{A^*K^*[CD]} \quad (1)$$

where $K$ is the binding constant, $I_0$ is the initial fluorescence intensity of flavonoids (without CDs), $I$ is the fluorescence intensity of flavonoids in the presence of CDs. *[CD]* is the concentration of the host molecule while $A$ is a constant.

### 2.3. Molecular modeling

Semi-empirical AM1 method was applied to examine the complex geometry and interaction details at the molecular level. Atomic charges of FIS and GER molecules and β-CDs were calculated using the B3LYP/6-31G(d) method and basis set by performing natural population analysis (NPA). Geometry optimization was carried out to an energy convergence of 0.05 kJ/mol with the Polak-Ribiere conjugate gradient algorithm. Molecular dynamics calculations were performed at AM1 level and TIP3P cage (15 Å × 15 Å × 15 Å, 517 water molecules) was