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3,6-diHydroxyflavone/bovine serum albumin interaction in cyclodextrin medium: Absorption and emission monitoring

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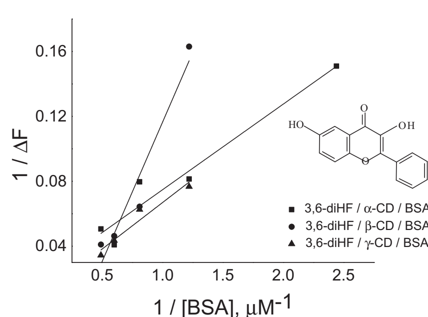
HIGHLIGHTS

- Photophysical properties of 3,6-diHF in CDs/BSA systems, were studied.
- The excited-state intramolecular proton transfer depends of the CDs cavity and BSA.
- The intrinsic fluorescence of BSA in the 3,6-diHF/BSA/CDs systems, is discussed.
- The use of 3,6-diHF as sensitive fluorescence probe in biological systems.

GRAPHICAL ABSTRACT



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ABSTRACT

Photophysical properties of a bioactive flavonol which can be used as a model for polyhydroxylated natural flavonols, 3,6-diHydroxyflavone (3,6-diHF) in cyclodextrins (CDs)/bovine serum albumin (BSA) systems have been studied by absorption and fluorescence spectroscopy. The influence of CDs nature and of the different molar ratios BSA/CDs on the fluorescent characteristics of 3,6-diHF, and on the excited – state intramolecular proton transfer (ESIPT) process were studied. Quantitative information on the interaction between 3,6-diHF and BSA in CDs medium, were estimated. The influence of temperature (25–60 °C range) on the intrinsic fluorescence of BSA in 3,6-diHF/BSA/CDs systems, was investigated. The results are discussed with relevance to 3,6-diHF as a potential sensitive fluorescence probe in the systems of biological interest.

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Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides with 6 (α-CD), 7 (β-CD) or 8 (γ-CD) glucose residues linked by a (1–4) glycosidic bond, having a conical molecular structure with a hollow interior of fixed volume [1,2]. These molecules are rigid and their outer surface is hydrophilic, while the internal cavity has non polar character in which a molecule or its functional group less hydrophilic than the solvent can be encapsulated, its dimension corresponding to that of the CDs cavity [1,2].

CDs are used as drug carriers to enhance the solubility, stability and bioavailability of the bioactive molecules, being of high level of biocompatibility, friendly to humans and approved by *Food and Drug Administration* [3–6]. To achieve biological activity, molecules have to reach the action site without losing the structural integrity, able to cross the lipophilic membrane [7,8].

Spectral studies on the inclusion complex with CDs showed that they are different from those of the guest molecule [9,10]. In these lines, it was reported that fluorescent characteristics of guest molecule are largely affected by inclusion complexation with CDs. That feature was attributed to the fact that inclusion of fluorophore in

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the cavity of CDs, places it in a more hydrophobic environment where it becomes protected from bulk solution quenchers [9,10].

Of importance is the effect of CDs on the interaction between several molecules of biological interest. Flavones are well known as related compounds of flavonoid group exhibiting intramolecular excited state proton transfer (ESIPT), dual fluorescence behavior and therapeutic properties [11–14]. In these lines, the spectrofluorometric study on the inclusion reaction of flavonols with β-CD, have been reported [15]. Here, the effect of β-CD on the acid dissociation equilibrium of the flavonols, inclusion constant and kinetics of inclusion reaction have been investigated in order to improve the selectivity and the sensitivity of flavonols in the spectrofluorometric determination of metal ions by addition of β-CD [15]. Spectroscopic and reactivity studies on kaempferol–CDs interaction [16] have been recently reported. Complex formation was monitored by two-dimensional ROESY experiments through the detection of intramolecular dipole interaction [16]. It was found that the B-ring of kaempferol is immersed in the apolar cavity with A- and C-ring protruding from the wider rim for the β-CD and its derivatives [16]. Studies on the encapsulation of 3-Hydroxyflavone (3-HF), fisetin and myricetin have been recently reported [17–19]. It was found that in the presence of β-CD, 3-HF and fisetin show significantly enhanced relative yields of the tautomer emission [17]. Also, these flavonols are encaged in predominantly hydrophobic micro-environments, where external hydrogen bonding perturbations (interfering with the intrinsic ESIPT), and dipolar relaxation effects, are minimized, influencing the binding affinity of the guest flavonols [17]. In γ-CD, enhancements in the intensity and anisotropy of the ESIPT tautomer fluorescence of 3-HF, are observed [18]. For myricetin, a facile entry into the cavity of β-CD, with a driving force of inclusion attributed to strong van der Waals interaction and formation of hydrogen bond between β-CD and myricetin, has been observed [19].

Recently it was reported that CDs offer the potential of modulating protein–surfactant interaction [20]. It was found that the presence of CDs can slightly hinder the strong interactions between bovine serum albumin (BSA) and anionic surfactant by the combination of electrostatic and hydrophobic interactions [20]. Also, the effectiveness of α-CD is lower than that of β-CD, due to the lower association constant between α-CD and surfactant [20].

According to the literature, proteins (including enzymes) are frequently the targets for therapeutically active flavonoid compounds of both natural and synthetic origin [21–23]. Studies on the interaction between flavonoids and serum proteins are important because serum proteins play a critical role in the transport and disposition of flavonoids, thus increasing their bio-availability [24]. BSA is the most abundant serum protein (the globular protein in plasma). It is a polypeptide chain of 582 amino acids residues, and has a relatively high aqueous solubility thus binding several types of biological molecules which play an important role in determining physiological function [25,26]. BSA has two Tryptophan (Trp) residues involved in its intrinsic fluorescence: Trp212 which belongs to subdomain IIA within a hydrophobic binding pocket and Trp134 which belongs to the first subdomain IB, located on the surface of the albumin molecule. In these lines, binding of various ligands is studied by means of fluorescence spectroscopy, monitoring the changes in the intrinsic fluorescence of BSA [27]. In this context, several spectroscopic studies on flavonoids–serum proteins interaction, have been reported [28–36].

This work follows our previous works [37–41] and deals with photophysical properties of a bioactive flavonol which can be used as a model for polyhydroxylated natural flavonols, 3,6-diHydroxyflavone (3,6-diHF) in systems based on cyclodextrins (CDs) and bovine serum albumin (BSA), studied by absorption and steady-state fluorescence spectroscopy. The influence of CDs nature, as well as of the different molar ratios protein/CDs on the fluorescent characteristics of 3,6-diHF, and subsequently on the excited – state intramolecular proton transfer (ESIPT) process were studied. The influence of temperature (range 25–60 °C) on the intrinsic fluorescence of protein (BSA) into the inclusion complexes 3,6-diHF/BSA/CDs, have been also investigated. The results are discussed with relevance to 3,6-diHF as a potential sensitive fluorescence probe in the systems of biological interest.

Experimental

Materials

The structure of the studied 3,6-diHydroxyflavone (3,6-diHF) is shown in Scheme 1. The stock solution of 3,6-diHF (5.66 mM) was prepared in methanol. Aliquots from stock solution (53 μl) were diluted with different concentration of cyclodextrins (CDs), α-, β- and γ-CD, and bovine serum albumin (BSA), to final working molar ratios of 1:1, 1:2, and 1:3 respectively. 3,6-diHF/BSA/CDs in different molar ratios, 1:1:1, 1:1:2 and 1:1:3 have been investigated. Methanol of spectrophotometric grade, was purchased from Sigma. The stock solutions of CDs (5 mM) and BSA (0.1 mM) were prepared in distilled water. The final working volume was 3 ml. BSA was purchased from Merck Darmstadt, Germany. α-, β-, and γ-CD were purchased from Aldrich, Merck and Sigma, respectively.

Methods and apparatus

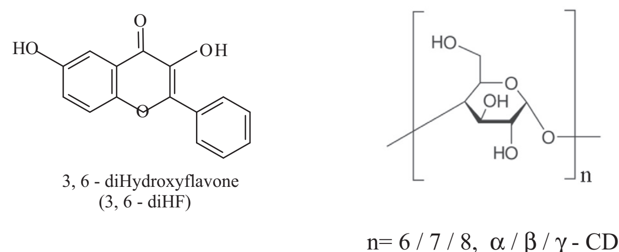
The absorption measurements were recorded using a Perkin Elmer, Lambda 35, UV–Vis Spectrometer at a scan rate of 480 nm/min and a spectral resolution of 1 nm.

The fluorescence emission and excitation spectra were recorded with a Jasco FP-6500 Spectrofluorometer, using 3 nm bandpasses for the excitation and the emission monochromators, the detector response of 1 s, data pitch of 1 nm, the scanning speed of 100 nm/min. The excitation wavelength was 365 nm, for flavone contribution and 280 nm, for Trp contribution.

Results and discussion

Absorption measurements

Fig. 1A presents absorption spectra of 3,6-diHF/CDs interaction, at a 1:1 M ratio in direct comparison with the absorption spectrum of 3,6-diHF in aqueous solution. It is observed that in aqueous solution, 3,6-diHF has three absorption bands: 257 nm, 336 nm and a broad band around 404 nm. This last one absorption band is attributed to the mono-anionic form of 3,6-diHF. As it can be seen, for a 1:1 M ratio of 3,6-diHF/CDs, the absorbance increases with a pronounced band at 329 nm in the case of 3,6-diHF/γ-CD. The same hypsochromic shifted band is observed for 3,6-diHF/α-CD while, no absorption band around 404 nm is observed. This feature



Scheme 1. Molecular structure of 3,6-diHydroxyflavone and CDs.

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