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Encapsulation of 3-hydroxyflavone in γ-cyclodextrin nanocavities: Excited state proton transfer fluorescence and molecular docking studies

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ABSTRACT

Steady state and time resolved fluorescence spectroscopy have been used to explore the confinement of 3-hydroxyflavone (3HF), (a bioactive flavonol) in γ-cyclodextrin (γ-CDx) nanocavities in aqueous medium. With increasing concentrations of γ-CDx, dramatic enhancements occur in the intensity and anisotropy of the excited state intramolecular proton transfer (ESIPT) tautomer fluorescence of 3HF. These observations indicate that 3HF readily enters the relatively hydrophobic cavity of γ-CDx, where the chromone ring is well shielded from external H-bonding perturbation effects, thus facilitates the ESIPT process. Additionally, appearance of induced circular dichroism (ICD) bands is noted in the absorption region of 3HF, which further confirms the inclusion process. Docking calculations suggest that hydrogen bonding interactions are involved in the formation of the inclusion complex.

1. Introduction

In 1936 Rusznyak and Szent-Györgyi first drew attention to the therapeutically beneficial role of flavonoids [1] which are polyphenolic compounds ubiquitous in higher plants [2,3] and widely present in plant based food and beverages (e.g. apple, broccoli, onion, soy products, tea, red wine, etc.). In recent years there has been a remarkable growth of research on various bioactive flavonoids which are effective against a wide range of free radical mediated human diseases (e.g. atherosclerosis, ischemia, neuronal degeneration, cancers, tumors, allergies, cardiac problems, inflammation, AIDS, etc.). High potency and low systemic toxicity of these compounds have made them viable alternatives to conventional therapeutic drugs [4–6]. However, such applications are often limited by poor water solubility which seriously restricts the bio-availability of these compounds. One of the possible ways for overcoming this is to increase aqueous solubility of the flavonoids by encapsulation in various drug delivery vehicles like liposomes and cyclodextrins. Cyclodextrins are cyclic oligomers comprised of α-D-glucose units (6, 7 or 8 α-D-glucose units and they are referred to α, β or γ-cyclodextrin respectively) and can be represented as a toroidal shape with a hydrophobic cavity (Scheme 1) with cavity diameter 0.45–0.8 nm [7]. This unusual shape of cyclodextrins enables confining a variety of small hydrophobic molecules thereby enhancing their solubility and stability in aqueous solution [8–10]. Moreover, the reduced polarity and restricted environment inside cyclodextrin cavities furnishes an interesting platform for studying various aspects of photophysical phenomena of the encapsulated molecules [11–13].

From a photophysical context, flavonols (3-hydroxyflavones, which represents the most common chemical class among naturally occurring flavonoids) have emerged as one of the best known molecular systems exhibiting excited state intramolecular proton transfer (ESPT) and 'two color' fluorescence behavior (Scheme 1) [14–17]. Thus 3-hydroxyflavone (3HF) and its derivatives can be used as exquisitely sensitive fluorescence probes for exploring binding sites in various bio-relevant targets e.g. proteins, DNA, bio-membranes and membrane mimetic organized assemblies (liposomes, normal and reverse micelles) [13,18–26]. In addition to its spectacular fluorescence properties, 3-HF was recently shown to exhibit significant antioxidant effects against membrane lipid peroxidation in red blood cell membranes as well as in model biomembranes (liposomes) [24,25]. Tormo et al. studied the interaction of 3HF with γ-cyclodextrin in non aqueous solvents based on UV–vis absorption and fluorescence emission studies and suggested formation of a caged anion of 3-HF in the cyclodextrin cavity [27]. However, to the best of our knowledge, till date, the mode

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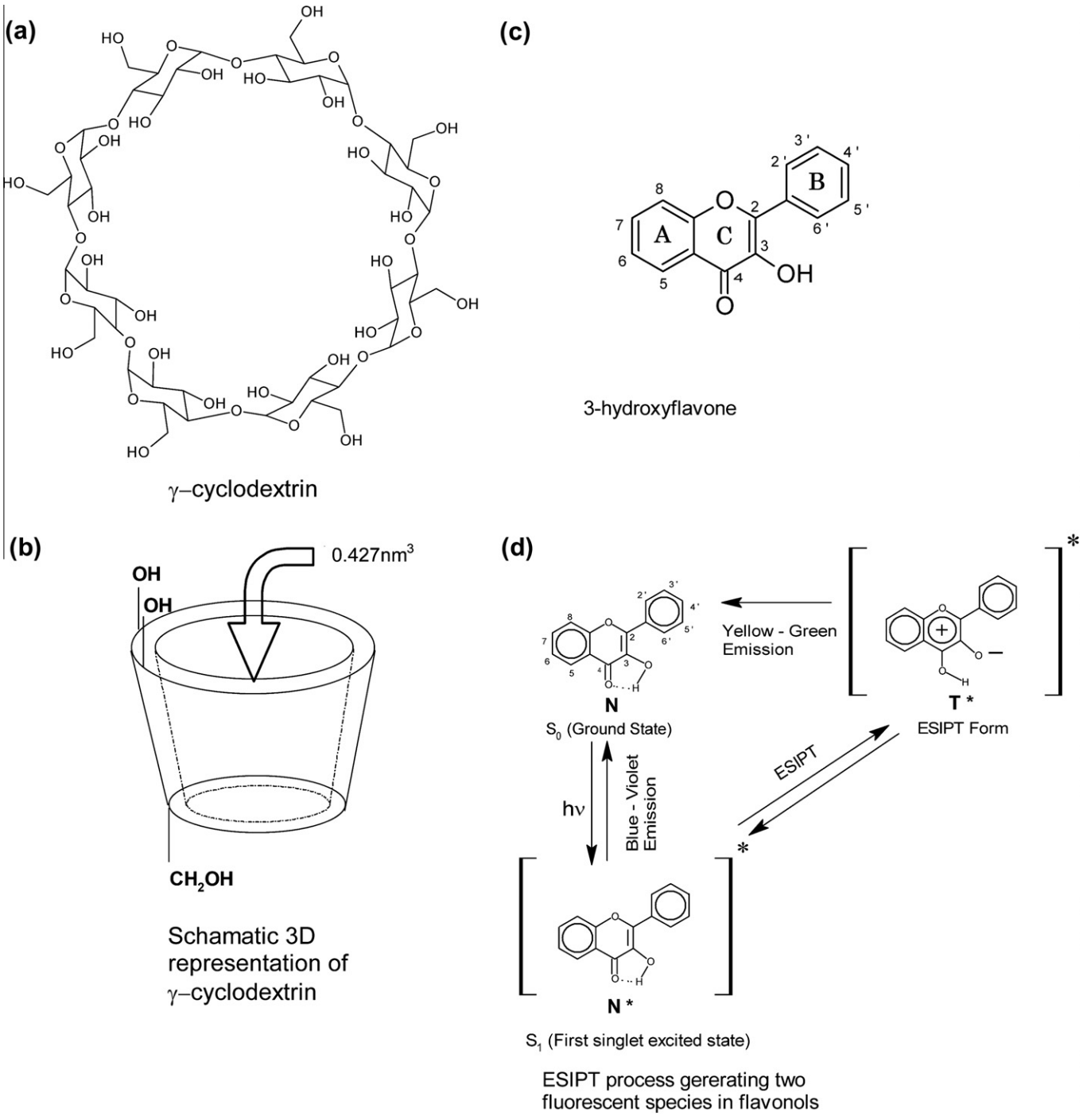

Scheme 1. Structure of γ-cyclodextrin and 3-hydroxyflavone (3HF) molecules. Intra molecular excited state proton transfer (ESIPT) in 3HF forming two different fluorescent species in photo excited state [14–16].

of inclusion as well as specific interactions of 3HF with γ-cyclodextrin in aqueous media, which is more relevant from physiological and pharmacological perspectives, are not known. Here, we have made qualitative as well as quantitative studies of the interactions of 3HF with γ-cyclodextrin in aqueous buffer using steady state fluorescence (dual emission profiles, intensity and anisotropy) and fluorescence decay analysis combined with induced circular dicroism (ICD) spectroscopy. Furthermore, we present for the first time, molecular modeling studies which provide detailed insights regarding the encapsulation process, including driving forces for the inclusion complex formation.

2. Materials and methods

2.1. Experimental

3-Hydroxyflavone was purchased from Aldrich Chemical Company, and was recrystallized twice from methanol. γ-Cyclodextrin was purchased from Sigma and used as such. Solvents used were of spectroscopic grade and checked for absorbing and fluorescent impurities. A concentrated stock-solution of 3HF was prepared in ethanol, from which requisite aliquots were added to γ-cyclodextrin solutions. The final concentration of 3HF was kept in the order