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Fluorescence properties of *trans*-ethyl-*p*-(dimethylamino) cinnamate in presence of bile acid host

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ABSTRACT

The knowledge of the formation of bile acid micellar aggregates is of great importance because of the biological significance of these compounds and their pharmacological applications. The intramolecular charge transfer (ICT) fluorescence property of *trans*-ethyl-*p*-(dimethylamino) cinnamate is used to study the micelles formed by aggregation of three most important bile acids, viz. cholic acid, deoxycholic acid and chenodeoxycholic acid by steady state and picosecond time-resolved fluorescence spectroscopy. The ICT fluorescence band intensity was found to increase with concomitant blue shift with the addition of bile acids. The blue shift in ICT fluorescence maxima as well as decrease in nonradiative decay constants in presence of bile acids indicate the passage of the probe towards the micro domains formed from the aggregated bile acids. Binding constant of the probe with micelles as well as critical micelle concentration and average polarity parameter of the micellar environments were obtained from the variation of fluorescence intensity on increasing concentration of bile acids in the medium.

1. Introduction

Research in the field of bile acids (BAs) has received considerable interest in recent times both from their biochemistry and physiology with the elegant supramolecular systems designed from them as well as their interesting physico-chemical behavior [1–3]. BAs are steroid acids found predominantly in the bile of mammals. Since BAs are made from endogenous cholesterol, the enterohepatic circulation of BAs may be disrupted as a way to lower cholesterol. This is the mechanism of action behind BA sequestrants. Bile acid sequestrants bind bile acids in the gut, preventing their re-absorption. In doing so, more endogenous cholesterol is shunted into the production of bile acids, thereby lowering cholesterol levels. The sequestered bile acids are then excreted in the feces.

In humans, the most important bile acids are cholic acid (CA), deoxycholic acid (DCA), and chenodeoxycholic acid (CDCA) (Fig. 1). The one or more α-oriented hydroxyl groups of BAs are put on the concave surface (α-face) of the steroid backbone and the methyl groups are positioned on the opposite convex side (β-face). Free molecules of BAs, normally cylindrical shapes of 20 Å long with a radius of about 3.5 Å, have a great surface activity and inclination to the formation of large aggregates, owing to this difference in orientation of hydrophilic and hydrophobic groups on the steroid ring systems. Prior to secretion by the liver, they are conjugated with either of the amino acids glycine or taurine. Conjugation further increases their water solubility, preventing passive re-absorption once secreted into the small intestine. As a result, the concentration of bile acids in the small intestine can stay high enough to form micelles and solubilize lipids. Study of formation of micelle of BAs is of great importance for understanding their interaction with biological membranes [4], bile secretion [5], solubilization of hydrophobic molecules such as cholesterol [6–8] and resveratrol [9] as well as for their roles as promoters of transport of some drugs through the intestine mucous membrane [10] etc.

The formation mechanism and structure of BA micelle as well as the cmc values were investigated by different spectroscopic techniques like NMR, EPR and CD [11–14], X-ray scattering [15], light dispersion and refractometry [16] and also by various physical methods like heat of solution and surface tension measurements [16,17], thermal titration and measurement of microviscosity [18,19], etc. In some of the recent publications, Pártay et al. reported molecular dynamics (MD) computer simulation method to study the aggregation process of the sodium salts of CA and DCA [20,21]. Such diversity of methods used in the study of BAs further confirms the great importance and existing interest in the study of these biological systems; partly because of their uniqueness in the structure when compared with long apolar tail – small polar head containing synthetic counterparts and, in part, due to their known importance in biological functions. For example, it has recently been reported that the promotory action of BAs in transportation of drugs in biological systems depends on their cmc values [22].

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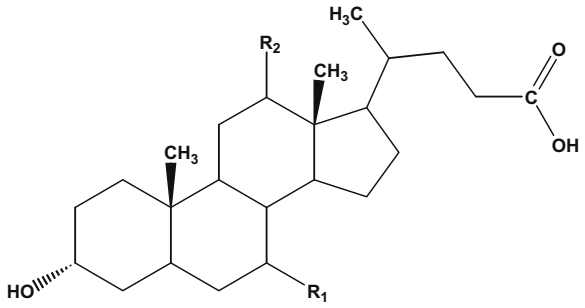

Bile acid (BA)	R_1	R_2
Cholic Acid (CA)	α-OH	α-OH
Deoxycholic acid (DCA)	H	α-OH
Chenodeoxycholic acid (CDCA)	α-OH	H

Fig. 1. Structure of bile acids (BA) used in this study.

Determination of cmc values of different micellar system is well studied by fluorescence probe method; although reports on probing the BA micellization by this technique are relatively scarce [23–28]. Nichifor et al. studied the aggregation behavior of BA modified dextran using N-phenyl-1-naphthyl amine as a fluorescence probe and reported that the cmc value depends on the nature of hydrophobic moiety and the degree of substitution [25]. Fluorescence probes like fluorescein isothiocyanate and also other polycyclic aromatic hydrocarbons having wide range of aqueous solubility were used to determine the cmc value of sodium taurocholate in water [26]. Matsuoka et al. used pyrene as the fluorescence probe to determine the cmc of sodium salt of DCA [27], whereas, Zhang et al. measured the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene to study the micellization of CA salt [28].

Fluorescence based techniques based on intramolecular charge transfer (ICT) phenomenon are quite common to study the physico-chemical properties of several systems, popularly known as organized media, which can compartmentalize solvents and solutes as well as sequester them from the bulk environment [29,30]. Surprisingly, most of the reports on ICT luminescence as well as different other photoprocesses in organized supramolecular assemblies are confined toward surfactants and cyclodextrin inclusion complexes [30–33]. Studies based on a completely different class of amphiphilic compounds, like BAs and their salts, as an alternative to the synthetic detergent or cyclodextrins to improve luminescence analysis are scantly observed [34,35]. Recently, it was reported that *trans*-ethyl-*p*-(dimethylamino) cinnamate (EDAC) shows ICT behavior upon excitation [36,37]. The low energy, unstructured, solvent dependent ICT emission of EDAC was used as an efficient reporter to study the microenvironment of micelles and cyclodextrins [38,39]. The fluorescence properties of EDAC show excellent correlation with polarity parameters. In this study, we report the fluorescence behavior of EDAC in presence of three BAs, viz., CA, DCA and CDCA by steady state and time-resolved fluorescence spectroscopy.

2. Experimental

trans-Ethyl-*p*-(dimethylamino) cinnamate (EDAC) was synthesized using standard procedure based on Reformatsky reaction [37]. The crude compound was purified by column chromatography and repeated crystallization. Further characterization was done by NMR and infra-red spectroscopy. CA, DCA and CDCA were all obtained from Sigma Aldrich Chemical Pvt. Ltd. (product no. C1129, D2510 and C9377, respectively) and used as received. The water used as solvent in all the measurements was obtained from Elix10 water purification system (Millipore India Pvt. Ltd.). All experiments were carried out at room temperature (293 K) with buffer solution of pH 9.2, freshly prepared by dissolving one buffer tablet (product no. 17351) obtained from Qualigens fine chemicals (a division of GlaxoSmithkline Pharmaceuticals Ltd.), India in 100 ml water [40].

Steady state absorption spectra were recorded on a Perkin–Elmer model Lambda25 absorption spectrophotometer. Corrected fluorescence spectra were taken in a Hitachi model FL4500 spectrofluorimeter. Quartz cuvettes of 10 mm optical path length received from PerkinElmer, USA (part no. B0831009) and Hellma, Germany (type 111-QS) were used for measuring absorption and fluorescence spectra, respectively. In both fluorescence emission and excitation spectra measurements, 5 nm bandpass was used in the excitation and emission side. Fluorescence quantum yields (ϕ_f) were calculated by comparing the total fluorescence intensity (F) under the whole fluorescence spectral range with that of 4-(dimethylamino) cinnamic acid, ($\phi_f^s = 0.002$ [40]) using the following equation:

$$\phi_f^i = \phi_f^s \cdot \frac{F^i}{F^s} \cdot \frac{1 - 10^{-A^s}}{1 - 10^{-A^i}} \cdot \left(\frac{n^i}{n^s}\right)^2 \quad (1)$$

where A^i and A^s are the optical density of the sample and standard, respectively, and n^i is the refractive index of solvent at 293 K. The relative experimental error of the measured quantum yield was estimated within ±5%.

The fluorescence decay curves in homogeneous buffer solution as well as in presence of bile acids were obtained using time correlated single photon counting (TCSPC) technique. Details of time-resolved fluorescence measurement are essentially the same as described elsewhere [41]. The excitation was done at 380 nm obtained by frequency doubling (GWU, Spectra Physics) of the tunable, picosecond version of a Ti:Sapphire laser (Tsunami 3950 Standard, Spectra Physics, Mountain View, CA) output in the range of 720–830 nm pumped by 5 W Nd-YLF laser (Millenia X, Spectra