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Encapsulation of serotonin in β-cyclodextrin nano-cavities: Fluorescence spectroscopic and molecular modeling studies

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ABSTRACT

Serotonin is a physiologically important biogenic amine, deficiency of which leads to mental disorders such as Alzheimer's disease, schizophrenia, infantile autism, and depression. Both β-cyclodextrin (β-CD) and its chemically substituted synthetic varieties (often possessing enhanced aqueous solubility and improved drug complexing abilities) are finding wide applications as drug delivery vehicles. Here we have studied the encapsulation of serotonin in β-CD and succinyl-2-hydroxypropyl β-cyclodextrin (SHP-β-CD) by exploiting the intrinsic serotonin fluorescence. Enhanced fluorescence emission intensity (which increases by ~18% and 34% in β-CD and SHPβ-CD respectively) and anisotropy (r) (r = 0.075 and 0.1 in β-CD and SHPβ-CD respectively) are observed in presence of the cyclodextrins. From the fluorescence data host–guest interaction with 1:1 stoichiometry is evident, the association constants (K) being 126.06 M^{-1} and 461.62 M^{-1} for β-CD and SHPβ-CD respectively. Additionally, molecular docking and semiempirical calculations have been carried out which provide, for the first time, detailed insights regarding the encapsulation process. In particular, it is evident that the indole ring is inserted within the β-CD cavity with the aliphatic amine side chain protruding towards the primary rim of the β-CD cavity. Docking calculations reveal that hydrogen bonding interactions are involved in the formation of the inclusion complex. Semiempirical calculations indicate that formation of the 1:1 inclusion complex is energetically favorable which is consistent with the fluorescence data.

1. Introduction

Serotonin (5-hydroxytryptamine or 5-HT) (Scheme 1a), is a biogenic amine found in many mammalian tissues as well as in lower organisms. Apart from acting as an important neurotransmitter, it has remarkable hormonal activities and is involved in smooth muscle contraction, stimulation of respiratory and circulatory chemoreceptors and nerve endings, as well as in various cognitive and behavioral functions including sleep, mood, pain, addiction, locomotion, sexual activity, anxiety, aggression, and learning [1,2]. Disruptions in serotonergic systems can lead to mental disorders such as Alzheimer's disease, schizophrenia, infantile autism and depression [3].

Serotonin is intrinsically fluorescent [4]. Kishi et al. [5] examined the fluorescence emission behaviour of serotonin in H_2O and D_2O at various pH values. Later on, detailed characterization of its photophysical properties and their modulation by ionization and polarity of the medium was reported by Chattopadhay et al. [4]. Ouyang and Vogel [6] explored the interactions of serotonin and melatonin (a structural analog of serotonin) with the calcium binding protein calmodulin (CaM) via fluorescence, far and near UV CD, NMR spectroscopy and other techniques. Such ventures [4–6] have prompted the present research aimed at detailed photophysical characterization of serotonin in cyclodextrins. Since serotonin is used in the therapy of depression and related diseases, its encapsulation in suitable drug delivery vehicles deserves detailed exploration. Furthermore, cyclodextrin–serotonin interaction is a model for enzyme active sites. The present research was undertaken in an attempt to assess the encapsulation of serotonin with β-cyclodextrins (chosen as drug delivery vehicles as well as a model for enzyme active sites) using its intrinsic fluorescence properties as well as via theoretical studies.

In this context, it is noteworthy that the fluorescence emission maximum (λ_{em}^{max}) of 5-hydroxyindole, which is the chromophore of serotonin, is insensitive to the surrounding environment which is generally attributed to the lack of appreciable solvent dipolar relaxation effects [7,8]. On the other hand, other steady state emission parameters, namely fluorescence intensity (F) and anisotropy (r) were found to be quite sensitive [7,8] to relevant environmental changes.

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a relatively less polar (compared to water) central cavity which are capable of encapsulating a wide range of or-

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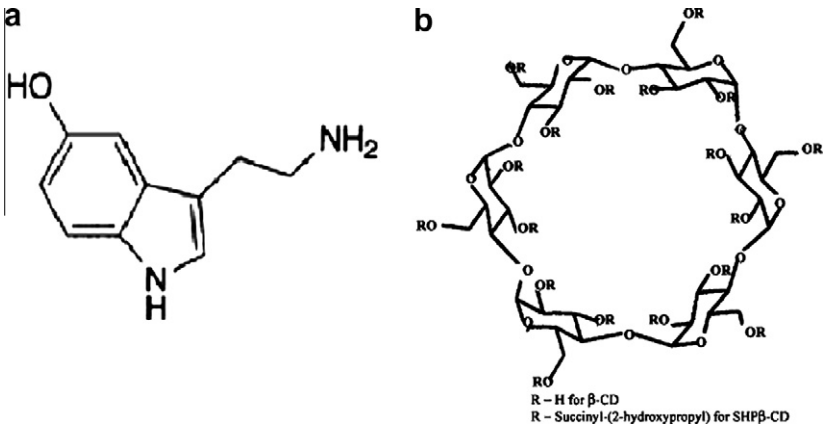

Scheme 1. (a) Serotonin. (b) β-cyclodextrin.

ganic and inorganic compounds [9–20]. In aqueous solutions they are able to form host–guest inclusion complexes with a variety of drug molecules. There is generally an improvement in the stability of the drug molecules upon such encapsulation [21–23]. It is this property of cyclodextrins that is making their use extremely popular as vehicles for drug delivery. Beta-cyclodextrins (β-CDs) (Scheme 1b) are the most readily available and widely used amongst the three cyclodextrins (CDs), namely alpha (α), beta (β) and gamma (γ) CDs, composed of 6, 7 and 8 α-D-glucose units, respectively. Although β-CDs have extensively been used as agents for drug delivery, recently synthetically derived substituted β-CDs are finding wider application because of their enhanced solubility in water and better drug complexing properties. The restricted space and relatively reduced polarity of the CD cavity influence the photophysical properties of guest molecules included in the cavity [24]. This has been utilized in the studies of inclusion complexation equilibria between CD and guest and the dynamics of the guest in the CD cavity. Bisby et al. studied single- and multi-photon excited fluorescence from serotonin complexed with β-cyclodextrin and suggested that hyperluminescence occurs from multiphoton excitation of a single isolated serotonin molecule [25]. However, to the best of our knowledge, till date, the mode of inclusion as well as specific interactions of serotonin with β-cyclodextrins are not known. Furthermore, there is no clear idea regarding the effects of chemical modifications of β-cyclodextrins on the complexation properties of serotonin. Here, we have made a qualitative as well as quantitative comparison of the interactions of serotonin with the β-cyclodextrin and its chemically modified derivative, SHPβ-cyclodextrin from fluorescence spectroscopic studies (fluorescence intensity and anisotropy). Furthermore, we present for the first time, theoretical studies (docking and semiempirical calculations) which provide detailed insights regarding the encapsulation process.

2. Materials and methods

2.1. Experimental

Serotonin, β-cyclodextrin (β-CD) and succinyl-(2-hydroxypropyl)-β-cyclodextrin (SHPβ-CD) [degree of substitution (DS) = 4] were purchased from Sigma. Solvents used were of spectroscopic grade and were preliminarily checked for absence of absorbing and fluorescent impurities. A stock solution of serotonin with a concentration of 2×10^{-3} M was prepared in methanol. Stock solutions of β-cyclodextrin and SHPβ-cyclodextrin both with a concentration of 1×10^{-2} M were prepared in quartz distilled water. To prepare each solution for spectroscopic measurements, an aliquot of the serotonin stock solution (in methanol) was transferred into a glass vial and the methanol was then evaporated. The dried samples were resuspended in specific volumes of β-cyclodextrin and SHPβ-cyclodextrin solutions and vortexed. The concentration of serotonin was maintained at 1×10^{-5} M in all the solutions. The samples prepared for spectroscopic measurements were then equilibrated at room temperature (25 °C) for 1 h prior to spectroscopic measurements. For emission measurements of the solutions of serotonin in cyclodextrin, background fluorescence as well as light scattering were removed by subtraction of the spectra on blank solutions.

Steady state fluorescence spectra were recorded with a Varian Cary Eclipse spectrofluorometer. The fluorescence readings were taken by exciting the samples and measuring the emissions, at appropriate wavelengths and appropriate water/buffer blanks were subtracted from respective measurements. A quartz cuvette of 1 cm path length was used in all experiments. For steady state fluorescence titrations, three independent measurements were performed. Values of binding constants are expressed as mean ± SD for three different experiments.

The fluorescence anisotropy (r) values were obtained using the expression

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad (1)$$

where I_{VV} and I_{VH} are the vertically and horizontally polarized components of probe emission with excitation by vertically polarized light at the respective wavelength and G defines the instrumental correction factor (polarization characteristics of the photometric system) calculated as [26]

$$G = \frac{I_{HV}}{I_{HH}} \quad (2)$$

Each intensity value used in this expression represents the computer-averaged values of ten successive measurements.

2.2. Theoretical studies

2.2.1. Docking study

We used Lamarkian genetic algorithm to identify the possible mode of serotonin binding mode in cyclodextrin nano-cavity. Serotonin structure was created using HYPERCHEM [27] molecular builder and optimised using AM1 force field to rms convergence of 0.01 kcal/Å mol with Polak-Ribiere conjugate gradient algorithm implemented in the HYPERCHEM 7.5 package and the β-cyclodextrin structure was extracted from the crystal structure of β-CD complex obtained from Protein Data Bank (PDB ID-3CGT). β-CD