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Study of interaction of proton transfer probe 1-hydroxy-2-naphthaldehyde with serum albumins: A spectroscopic study

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Abstract

In the present work, we have studied the interaction of proton transfer probe 1-hydroxy-2-naphthaldehyde (HN12) with Human Serum Albumin (HSA) and Bovine Serum Albumin (BSA) by steady state absorption and emission spectroscopy combined with time resolved fluorescence measurements. The measured binding constant (K) and free energy change (ΔG) indicate a stronger affinity of HN12 molecule for HSA than BSA. Steady state anisotropy, excitation anisotropy and fluorescence resonance energy transfer (FRET) studies indicate that the probe molecule resides at the hydrophobic site of the protein environment.

Keywords: 1-Hydroxy-2-naphthaldehyde; Serum albumins; Absorption; Fluorescence emission; Fluorescence resonance energy transfer

1. Introduction

Serum albumins (HSA and BSA) are found to be major protein components of blood plasma [1,2]. They serve as physiological carriers for fatty acids [3], lysolecithin [4], bilirubin [5] and bile salts [6]. These proteins are also capable of binding with metals [7], hormones [8] and an impressive variety of therapeutic drugs [9]. Therefore, the binding of different categories of small molecules with serum albumins is interesting for studying binding mechanism, protein structure and dynamics [10,11]. Study of these proteins has gained attention of many research groups all over the world- among bioscientists, chemists and therapeutists. These are homologous globular proteins composed of single polypeptide chains with 583 and 585 amino acid residues for BSA and HSA, respectively [12]. The tertiary structure of the protein is composed of three domains, and at least six binding sites are available for the binding of the ligands. Hence, the nature of binding of a ligand with HSA and BSA is different for different ligands [12,13].

Several methods have been developed for the detection of proteins in solution phase such as Lowry method [14], bicinchoninic acid [15] and Bradford method [16], and fluorescence spectrometry [17,18]. It is seen that fluorescence spectroscopy serves as an effective and cheaper method for such studies [17,18]. The only requirement for such study is to find a probe with relatively high quantum yield, capable of binding with protein molecules and fluorescence properties of the probe should change upon binding to proteins [17,18]. Thus, new extrinsic probes are designed to serve as reporters of the proteinous microenvironment [18–22]. These probe molecules can efficiently serve as reporter of the physiological activities in living systems and any extrinsic fluorescence probes can increase the benefit of fluorescence spectroscopy for studying protein structure and dynamics. The disadvantage for the use of such fluorescence probes is that there are several binding sites on each protein which makes the origin of fluorescence measurement more complex.

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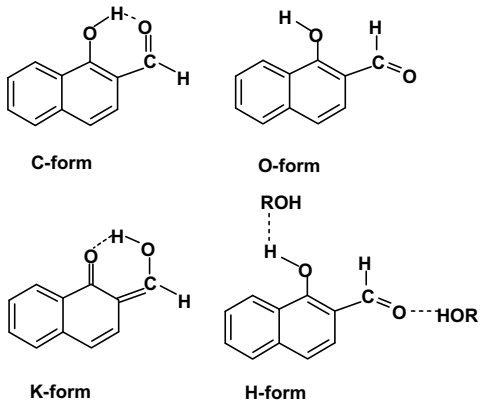
The phenomenon of charge, proton and electron transfer in the excited state of molecules is being widely investigated for their vast applications in the field of biochemistry and these molecules are efficient fluorescence sensors of the surrounding microenvironments [17–24]. The emissive properties of such molecules undergo noticeable changes with variation of surrounding environment and hence the change in spectral characteristics can help to predict the type of environment that surrounds the molecule. The excited state intramolecular proton transfer (ESIPT) probes have been successfully used for investigation of proteinous environment earlier [23,24].

Recently, the ESIPT reaction has been studied in a number of interesting organic molecules by our research group [25–27]. In the present work, we have investigated the changes in the photophysical behaviour of proton transfer probe 1-hydroxy-2-naphthaldehyde (HN12) due to binding with the HSA and BSA protein molecules. In general, large dye molecules (e.g. Nile red), pigments (e.g. Curcumin) and charge transfer molecular systems are used as reporter of the protein microenvironment to study protein structure and dynamics [11,20,22]. Here, we are interested in such study with a simple synthetic small proton transfer probe molecule. The photophysical study of HN12 in different solvents using steady state and time resolved fluorescence techniques has been carried out to establish the phenomenon of ESIPT process before pursuing this work [26]. The interaction of HN12 with the proteins leads to changes in spectral properties both in the ground as well as in the excited state. The shift of absorption and emission band positions, change of emission intensity and time resolved study provide insight into the binding nature of probe with protein molecules. Binding constant and free energy change are determined for both the HSA and BSA protein. Steady state anisotropy studies, excitation anisotropy and FRET studies have been purused to know the probable location of the probe molecule inside the protein environment.

2. Materials and method

The molecule HN12 (Scheme 1) was synthesized using simple literature procedure [28]. In brief, methyl iodide was added to a stirred and heated mixture of 1-hydroxy-2-naphthoic acid and K_2CO_3 and refluxed for 11 h. The brown colour liquid product of 1-methoxy-2-naphthyl-methyl ester was extracted with ether and then $LiAlH_4$ was added and stirred for 4 hours. In the next step the product was decomposed with Na_2SO_4 and oxidized by MnO_2 to produce 1-methoxy-2-naphthaldehyde. Treatment of 1-methoxy-2-naphthaldehyde with $AlCl_3$ in dichloromethane produced 1-hydroxy-2-naphthaldehyde (Scheme 1). The compound was purified by column chromatography and repeated crystallization. The Tris–HCl buffer of pH 7 was prepared from Tris buffer purchased from Sisco Research Laboratory (SRL), India. Bovine Serum Albumin from SRL, India and Human Serum Albumin form Aldrich Chemicals were purchased for preparing



Scheme 1. Different ground state structural isomers of HN12 and its hydrated clusters.

different solutions. Triple distilled water was used for the preparation of buffer solutions.

The absorption and emission measurements were done by Hitachi UV–Vis U-3501 spectrophotometer and Perkin–Elmer LS-50B fluorimeter, respectively. In all measurements, the probe concentration was maintained at 9.0×10^{-6} mol/dm^3 and the absorbance value was maintained below 0.50 in order to avoid aggregation and reabsorption effects.

Fluorescence quantum yield (Φ_f) was determined using the following equation where quinine sulphate is used as primary standard ($\Phi_f = 0.54$ for quinine sulpahte in 0.1 N H_2SO_4 [22]:

$$\frac{\Phi_S}{\Phi_R} = \frac{A_S}{A_R} \times \frac{(\mathrm{Abs})_R}{(\mathrm{Abs})_S} \times \frac{n_S^2}{n_R^2} \quad (1)$$

where Φ_S and Φ_R are the quantum yields, A_S and A_R are the integrated fluorescence areas, $(\mathrm{Abs})_S$ and $(\mathrm{Abs})_R$ are the absorbance values for the probe and reference molecule, respectively. n_S and n_R are the refractive indices of the medium for the probe and reference molecule, respectively. Steady state anisotropy measurements were carried out using the same fluorimeter. The steady state anisotropy r is defined as

$$r = (I_{VV} - G.I_{VH})/(I_{VV} + 2G.I_{VH}) \quad (2)$$

$$G = I_{HV}/I_{HH} \quad (3)$$

where I_{VV} and I_{VH} are the emission intensities when the excitation polarizer is vertically oriented and the emission polarizer is oriented vertically and horizontally, respectively. I_{HV} and I_{HH} are the emission intensities when the excitation polarizer is horizontally oriented and the emission polarizer is oriented vertically and horizontally, respectively and G is the correction factor.

A picosecond diode laser with 408 nm light (IBH, UK, NanoLED-07, s/n 03931) is used as exciting source for the picosecond TCSPC setup [29]. The fluorescence signal was detected in 54.7° magic angle polarization using Hamamatsu MCP PMT (3809U). The typical system response of the laser system is 90 ps. The decays were analyzed using