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Location and binding mechanism of an ESIPT probe 3-hydroxy-2-naphthoic acid in unsaturated fatty acid bound serum albumins

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ABSTRACT

The binding site and the binding mechanism of 3-hydroxy-2-naphthoic acid (3HNA) in oleic acid (OA) bound serum albumins (bovine serum albumin (BSA) and human serum albumin (HSA)) have been determined using steady state and time resolved emission of tryptophan residues (Trp) in proteins and the ESIPT emission of 3HNA. Time resolved anisotropy of the probe 3HNA and low temperature phosphorescence of Trp residues of BSA in OA bound BSA at 77 K reveals a drastic change of the binding site of 3HNA in the ternary system compared to that in the free protein. 3HNA binds near Trp213 in the ternary system whereas 3HNA binds near Trp134 in the free protein. The structure of OA bound BSA generated using docking methodology exhibits U-bend configuration of all bound OA. The docked pose of 3HNA in the free protein and in OA bound albumins (ternary systems) and the concomitant perturbation of the structure of proteins around the binding region of 3HNA corroborate the enhanced ESIPT emission of 3HNA and the energy transfer efficiency from the donor Trp213 of BSA to 3HNA acceptor in 3HNA–OA–BSA system.

1. Introduction

The studies on excited state intramolecular proton transfer reactions (ESIPT) are of great importance to understand many chemical and biological reactions [1–11]. The ESIPT reaction leads to the proton transfer from a hydroxyl (or amino) proton to the carbonyl oxygen (or pyridine nitrogen) with a femto second rise time [3–5] through a pre-existing hydrogen bonding (HB) configuration. This result is due to a particular tautomeric form having vast differences in structure and electronic configuration from its corresponding normal species. The tautomeric form shows unusual photophysical property and provides diverse excited state environmental responses [1,2]. The ESIPT emission of several flavonols [1] and their use as protein binding site fluorophore probes have studied by Kasha et al.

3-Hydroxy-2-naphthoic acid (3HNA) exhibits dual emission: a localized $^1(\pi-\pi^*)$ emission around 400–420 nm regions and an ESIPT emission around 505–510 nm regions from a rotamer [12–14]. Neutral 3HNA remains in equilibrium with its anionic form ($pK_a = 4$) in the ground state [12,15,16] (Scheme 1). 3HNA is a versatile probe since the nature of the emitting species depends on the concentration, pH of the medium, nature of the solvent and temperature [12,17]. The ESIPT emission of 3HNA at a low concentration of 20 μM in water medium (pH7) is found to be very weak. The ESIPT emission has been well characterized in various solvents [10,11]. In polar solvents the '$\pi\pi^*$' emission is weak, whereas the broad ESIPT band is enhanced [11]. 3HNA shows only the '$\pi\pi^*$' emission in nonpolar solvents like toluene and dichloromethane [10–11]. In a previous work we noticed that the ESIPT emission of 3HNA at a low concentration of 20 μM is significantly enhanced in aqueous medium due to binding with several albumins viz bovine serum albumin (BSA) and human serum albumin (HSA) [10]. The enhanced ESIPT emission of 3HNA in water soluble polymer Polyvinylpyrrolidone (PVP) was ascribed to absorption of 3HNA in an environment restricting the rotational freedom of 3HNA [18].

It is well known that serum albumins bind with different saturated and unsaturated fatty acids [19–34]. In 1941, Kendall observed that crystalline serum albumin always contains a small amount of free fatty acid that could not be removed by repeated crystallization [27]. Since then the binding nature and the effect of the binding of various saturated and unsaturated fatty acid to albumins are the important subject for investigations. From the electrophoretic studies it was shown that the mobility of the serum albumin increases in the presence of fatty acid salts [28,29]. It was also found that fatty acid anions stabilize serum albumin against denaturation by urea, guanidine and heat [30,31]. A trace amount of fatty acid could prevent the growth factor for tubercle bacilli and serum albumin also could protect sheep crythrocytes against hemolysis by oleic acid [32]. Fatty acids in general bind strongly

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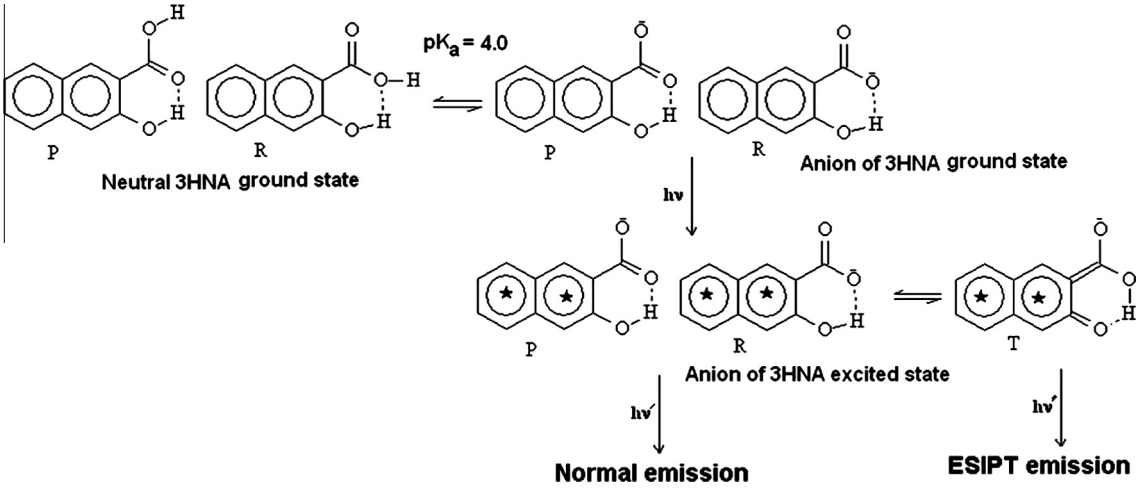

Scheme 1. Structural representation of 3HNA in its neutral and anionic forms in the ground and excited states.

with serum albumins and compete effectively with organic ligands for binding sites of albumin [19,33,34].

Although the steady state and time resolved fluorescence and anisotropy provide information regarding the binding of ligand, phosphorescence spectra of Trp residues in proteins and protein–ligand complex in a suitable cryosolvent at 77 K often reveal the specific perturbation of Trp residues. Trp phosphorescence in proteins is characterized by well structured spectra with narrow (0,0) band in contrast to broad band of Trp fluorescence [35–38]. The position, the width and the lifetime of the (0,0) band provide information regarding the environment of Trp residues. The blue shifted (0,0) band corresponds to Trp in polar and solvent exposed region whereas the red shifted (0,0) band is indicative of Trp in hydrophobic and buried environment [35,39,40]. The width of the (0,0) band reflects the homogeneity or heterogeneity around the Trp residue. The narrow width indicates more homogeneous environment [19]. Overlapping phosphorescence spectra from different Trp residues with different multiple (0,0) bands in multitryptophan proteins are observed when the singlet–singlet (S-S) nonradiative energy transfer (ET) between the Trp residues is prevented and the Trp residues have distinctly different environment in the protein [36,41–62].

In this paper we address the binding of the ESIPT probe 3HNA in fatty acid bound albumins to address the difference in the binding mechanism and binding location in the ternary system compared to the binding of the ligand to the free proteins. We have chosen the unsaturated oleic acid (OA) as the fatty acid since the crystal structure of OA bound HSA (HSA–OA) [22] is known and the binding of OA to BSA is known [19,63].

The binding of 3HNA in OA bound albumins has been characterized by (i) steady state and time resolved studies monitoring Trp fluorescence in albumins and also the ESIPT emission of 3HNA (ii) time resolved anisotropy decay of 3HNA in the binary (3HNA–BSA/HSA system) and in the ternary systems (3HNA–OA–BSA/HSA system). Low temperature (77 K) phosphorescence of free albumins, OA bound albumins, the complexes of 3HNA-albumins and the complexes of 3HNA with OA bound albumins (ternary system) have been successfully employed to find the location of 3HNA in the ternary system. Although the free BSA exhibits only one (0,0) band corresponding to tryptophan residue at 213 (Trp213), OA bound BSA exhibits two (0,0) bands in the phosphorescence spectra corresponding to Trp134 and Trp213 residues [19]. We exploited the optical resolution of two Trp residues in BSA–OA complex to find the perturbation of individual Trp residue due to the binding of 3HNA in the OA bound BSA.

Although the binding patterns of 3HNA in OA bound HSA is found to be slightly changed compared to the binding of 3HNA to free HSA, the binding site of 3HNA has been drastically changed in the ternary system of BSA (BSA–OA–3HNA) compared to that in the binary system of 3HNA–BSA. The crystal structure of HSA–OA is known where seven OA bind with HSA at different locations [22]. Since the structure of BSA–OA is not known, the BSA–OA structure has been generated by docking studies on the basis of our experimental composition of BSA–OA complex where six OA bind with BSA. Details of docking studies of the binary systems (3HNA–BSA/HSA) and the ternary systems (3HNA–OA–BSA/HSA) have been successfully exploited to corroborate our experimental results regarding the binding site of 3HNA and the concomitant perturbation of various residues in the fatty acid bound proteins. The energy transfer (ET) efficiency and the rate constants of ET from Trp residue to the bound 3HNA in the ternary system have also been explored.

2. Experimental section

2.1. Materials and methods

All chemicals were of reagent grade, which were used without further purification unless otherwise mentioned. The 3-hydroxy-2-naphthoic acid (3HNA), oleic acid (OA), serum albumin from bovine (BSA) and serum albumin from human (HSA) were purchased from Sigma–Aldrich, USA. The 3HNA was purified in EtOH by repeated crystallization. Phosphate buffer of pH7 was prepared in triply distilled water and used for making experimental solutions. Oleic acid is first dissolved in ethanol and then it is used to prepare the experimental solutions in aqueous medium. The final ethanol concentration in the solutions did not exceed 2% and had no effect on the measurements [19].

2.2. Instrumentation

The steady state emission measurements were carried out using a Hitachi Model F-7000 spectrofluorimeter equipped with a 150-W xenon lamp, at 298 K using stoppered cell of 1 cm and 0.5 cm path length for the measurement in phosphate buffer of pH7. The measurements of the Trp emission of proteins in all the samples were made by exciting the samples at 290 nm. The emission of 3HNA was observed by exciting the samples at 350 nm. In all the measurements we used the correct mode of the instrument and the inner filter effect has been eliminated. In both the