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Effect of the binding interaction of an emissive niacin derivative on the conformation and activity of a model plasma protein: A spectroscopic and simulation-based approach

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ABSTRACT

The present work demonstrates a detailed photophysics of bio-active drug-like acid viz., 2-hydroxynicotinic acid (2-HNA) and its interaction with a model plasma protein Bovine Serum Albumin (BSA). The drug which is in essence a vitamin-B_3 derivative, is capable of exhibiting ultrafast lactim-lactam cross-over response and thereby the modulation of the lactam emission within the bio-environment of the protein has been depicted spectroscopically to reveal the drug-protein interaction. Apart from evaluating the binding constant, the probable location of the neutral drug molecule within the protein cavity (hydrophobic subdomain IIIA) has been explored by AutoDock-based blind docking simulation technique. In this microheterogeneous medium, slow solvent reorientation time with respect to the emissive lifetime of the drug explicate the Red Edge Effect (REE). To complement the findings about the binding process, chaotrope-induced protein denaturation has also been inspected. The probe also illustrates a perceptible difference in rotational relaxation time in confined medium than in aqueous medium which strengthen our verdict. Unfolding of the protein in the presence of the drug molecule has been probed by the decrease of the α-helical content, obtained via circular dichroism (CD) spectroscopy, which is also supported by the gradual slaughter of the esterase activity of the protein in the presence of the drug molecule.

1. Introduction

Interactions between biomolecules, especially plasma proteins and small bio-compatible molecules have been a fascinating research topic in chemistry, life sciences and clinical medicine. The spatial orientation and altered properties of chemical entities entrapped within both the biological and biomimicking assemblies have garnered enormous attention compared to that in the pure homogeneous media [1–5]. Knowledge of the nature, mechanism and the dynamics of interactions between small molecules and proteins and the dynamics of the involved processes have vital importance to delve into the biochemical consequences of drug–protein interactions [4–7].

Serum albumins are the most abundant circulatory proteins in blood plasma (accounting for ~60% of the total blood plasma) and play a pivotal role by nonspecifically binding to several hydrophobic ligands as well as to carry them towards the target sites [7–10]. The extensively exploited serum albumin BSA contains two tryptophan residues, Trp-134 and Trp-212, of which the former is located in hydrophilic Subdomain IB, and it is proposed to be located near the surface of the albumin molecule in the second helix of the first domain while Trp-212 is located in hydrophobic Subdomain IIA [7–10]. According to the predictable view based on Sudlow's classification, the hydrophobic pockets at Subdomains IIA and IIIA are the principal regions of ligand interactions (the latter having the highest affinity). Some studies of binding of small molecules to albumin indicate that the hydrophobic interactions may be stronger than the eletrostatic forces, but the eletrostatic interactions are significant since uncharged hydrocarbon chains have lower binding affinity to the protein [11–14].

The drug-protein complex can thus be regarded as a form of the temporary store within the bio-medium; which can effectively avoid drug elimination via metabolism and thus can maintain its effective concentration in plasma. Consequently, interaction of a drug with, and competition for, the binding sites on plasma proteins might strongly affect its distribution, elimination as well as its pharmacodynamics and toxic properties [15–19]. 2-Hydroxynicotinic acid (2-HNA), a simple but unexercised Nicotinic acid (vitamin B_3 or niacin) derivative grabbed our attention in this context. Niacin is primarily used in the treatment of hypercholesterolemia (high cholesterol) and pellagra (niacin deficiency). Insufficient niacin in the diet can cause nausea, skin and mouth lesions, anemia, headaches, tiredness and so forth and therefore we anticipate this particular molecule to be bio-compatible and pharmaceutically relevant occurring with the molecular skeleton enabling us to follow its biochemical consequences in vitro by means of simple

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fluorescence measurements [18–20]. Lipid bilayer partitioning and permeation of this drug-like acid [21] in liposomal systems consisting of phosphatidylcholine alone or mixed with cholesterol or charged lipids augment its biological significance [21].

Keeping in mind these significances, the present program delineates the binding interaction of 2-hydroxynicotinic acid (2-HNA), with the model transport protein BSA on the milieu of the modulation in the photophysical properties of the probe within the constrained bio-environment of the protein. Other than fluorescence, the effect of the binding of this potential drug molecule on the conformation of the native protein has been explored using various other spectroscopic techniques e.g., circular dichroism (CD) spectroscopy and esterase-like activity to yield consensus results and the probable binding location of the drug within the microheterogeneous cavity has been unveiled from AutoDock-based blind docking simulation.

2. Experimental

2.1. Materials

Commercially available 2-hydroxynicotinic acid (2-HNA, vide Scheme 1) was obtained from Alfa Aesar, Lancester, and used as received. The purity of the compound was established on a TLC plate before use. BSA from Sigma Chemical Co., USA was used as received. Tris-HCl buffer was purchased from SRL, India, and 0.01 M Tris-HCl buffer of pH 7.4 was prepared in triply distilled deionized water from a Milli-Q water purification system (Millipore). The solvent appeared visually transparent and the purity was also checked by running the fluorescence spectra in the studied wavelength range. Urea for protein denaturation from E-Merck was used after recrystallization from methanol (AR Grade, E-Merck) twice. *p*-Nitrophenylactetate (PNPA) of Analytical Grade and Tween-20 surfactant were obtained from SRL, India and used without further purification.

2.2. Instrumentation and Methods

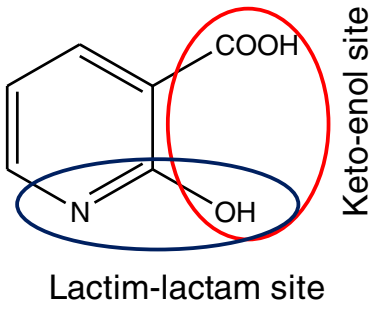
2.2.1. Steady-state Spectral Measurements

The absorption and emission spectra were recorded on a Hitachi UV–Vis U-3501 spectrophotometer and Perkin-Elmer LS55 fluorimeter, respectively, with suitable corrections for instrumental response. The recorded spectra were appropriately background subtracted with blank aqueous buffered solution in order to eliminate any spectral interference. Experiments have been carried out at an ambient temperature of 298 K, unless otherwise specified. Only freshly prepared solutions were used for spectroscopic measurements. The steady-state anisotropy (r) is defined as

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

where, I_{VV} is the fluorescence intensity when both the excitation and emission polarizers are oriented vertically, and I_{VH} is the fluorescence intensity when the excitation polarizer is vertically and the emission polarizer is horizontally oriented. The correction factor, G is given by

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



Scheme 1. Structure of 2-hydroxynicotinic acid (2-HNA).

2.2.2. Time-resolved Fluorescence Decay Measurements

Time-resolved fluorescence decays were obtained by the method of Time Correlated Single-Photon counting (TCSPC) on FluoroCube-01-NL spectrometer (Horiba Jobin Yvon) with the help of a light source (nanoLED) of 340 nm (at $\lambda_{exc} = 336$ nm) and the signals were collected at the magic angle of 54.7° to eliminate any considerable contribution from fluorescence anisotropy decay [4,5,7,14,18,19]. The typical time resolution of our setup was ~800 ps. The decays were deconvoluted on DAS-6 decay analysis software judging the acceptability of the fits by χ^2 criteria (fitting analyses having χ^2 values within the range 0.9–1.1 has only been retained) and visual analysis of the residuals of the fitted function to the data. Mean (average) fluorescence lifetimes ($<\tau_f>$) were calculated using the following equation:

$$<\tau_f> = \alpha_1\tau_1 + \alpha_2\tau_2 \quad (3)$$

in which α_1 and α_2 are the pre-exponential factors corresponding to the ith decay time constant, τ_i. For time-resolved fluorescence anisotropy decay measurements [4,5,7,19]. The time-resolved fluorescence anisotropy decay function, r(t) is given by

$$r(t) = \frac{I_{VV}(t) - GI_{VH}(t)}{I_{VV}(t) + 2GI_{VH}(t)} \quad (4)$$

where, $I_{VV}(t)$ and $I_{VH}(t)$ designate fluorescence decays obtained for parallel and perpendicular emission polarizations, respectively, with respect to the vertical excitation polarization [4,5,7,19].

2.2.3. Circular Dichroism Spectroscopy

Circular dichroism (CD) spectra were recorded on a JASCO J-815 spectropolarimeter using a cylindrical cuvette of path-length 0.1 cm at 25 °C. The reported CD profiles are an average of four successive scans obtained at 20 nm/min scan rate with appropriately corrected baseline. The concentration of BSA and the drug during CD measurements are mentioned in the relevant discussion.

2.2.4. Esterase Activity Assay

The effect of the drug on the esterase activity of BSA was assayed with the synthetic substrate *p*-nitrophenyl acetate (PNPA) by following the formation of *p*-nitrophenol [18,19] at 37 °C.

2.2.5. Molecular Modeling: Docking Study

The native structure of BSA was taken from the Protein Data Bank having PDB ID: 3V03 [22]. Docking studies were performed with AutoDock 4.2 suite of programs which utilizes the Lamarckian Genetic Algorithm (LGA) implemented therein. For docking of the drug with BSA, the required file for the ligand was created through the combined use of Gaussian 09W [23] and AutoDock 4.2 [24] software packages. The appropriate geometry of the molecule was first optimized at DFT// B3LYP/6-311++G(d,p) level of theory using Gaussian 09W suite of programs and the resultant geometry was read in AutoDock 4.2 software in compatible file format, from which the required file was generated in AutoDock 4.2. The grid size was set to 126, 126, and 126 along *X*-, *Y*-, and *Z*-axis with 0.419 Å grid spacing, i.e. in order to recognize the binding site of 2-HNA in BSA, the strategy of blind docking simulation was adopted. The AutoDocking parameters used were as follows: GA population size = 150; maximum number of energy evaluations = 250,000; GA crossover mode = two points. The lowest binding energy conformer was searched out of 10 different conformations for each docking simulation and the resultant minimum energy conformation