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Binding interaction between plasma protein bovine serum albumin and flexible charge transfer fluorophore: A spectroscopic study in combination with molecular docking and molecular dynamics simulation

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ABSTRACT

Binding interaction of plasma protein bovine serum albumin (BSA) with external flexible charge transfer fluorophore 5-(4-dimethylamino-phenyl)-penta-2,4-dienenitrile (DMAPPDN) has been explored at physiological pH (7.4) by steady state absorption, emission, fluorescence anisotropy, Red Edge Excitation Shift (REES), far-UV circular dichroism (CD), time resolved spectral measurements in combination with molecular docking and molecular dynamics (MD) simulation studies. Chemical denaturation of the protein bound probe by guanidine hydrochloride (GdnHCl) has also been tracked using the spectral response of DMAPPDN. Interaction of the probe with BSA is reflected by the massive blue shift of the fluorophore emission maxima (78 nm) with the enhancement of fluorescence intensity due to change of hydrophobic micro-environment of the probe compared to a little change in protein secondary structure. Benesi–Hildebrand plot reveals spontaneous formation of 1:1 BSA–DMAPPDN complex with binding constant $8.821 \pm 0.01 \times 10^3\ M^{-1}$ and binding free energy change -5.359 kcal mol^{-1}. Molecular docking study supports the binding of probe in the hydrophobic cavity of sub domain IIA of BSA. The distance for energy transfer from tryptophan of BSA to DMAPPDN measured from fluorescence resonance energy transfer (FRET) results is in good agreement with results of molecular docking study. MD simulation predicts greater stability of BSA–DMAPPDN complex compared to the free protein.

1. Introduction

Serum albumins are major transport proteins [1,2] found in the blood plasma and are capable of binding, transporting and delivering an extraordinarily diverse range of endogenous and exogenous compounds like fatty acids, nutrients, steroids, certain metal ions, hormones and a variety of therapeutic drugs [3–5] in the bloodstream to their target organs [6]. Although a large volume of research work is already established in diverse fields with BSA as a model protein, but till date, there exists an intriguing mystery regarding the various types of hydrophobic and hydrophilic interactions within the protein interior in the presence and absence of small molecules like drugs and/or fluorescent probes, since, the exact crystal structure of BSA is still unknown. The crystal structure of human serum albumin (HSA) is however well established. The pair wise sequence alignment has only one gap over all the residues of the BSA sequence with 75% identity and 87% similarity shared between human and bovine sequences [7]. The primary structure of BSA is a sequence of 583 amino acid residues and the secondary structure contains 67% alpha helix with six turns and 17 disulphide linkages [4,8–10]. The tertiary structure is formed by three homologous domains I→III, each of which is divided into two sub domains A and B [4,11]. Two tryptophan residues Trp-134 and Trp-213 are present in BSA in the IB and IIA sub domains, respectively [12–14]. This protein also contains two principal drug binding sites, site-I and site-II. Site-I is situated in the hydrophobic core of sub domain IIA which is capable of binding mostly with neutral, bulky heterocyclic compounds by strong hydrophobic interactions, whereas site-II is in the IIIA sub domain and binds many aromatic carboxylic acids by dipole–dipole, van der Waals and hydrogen bonding interactions [15]. The structural aspects of serum albumins and their properties and interactions with other materials have been explored by several groups [16,17] using NMR, dynamic light scattering, differential scanning calorimetry, circular dichroism and other techniques. In the last few years, in the field of photochemistry and photobiology, the intramolecular charge transfer (ICT) fluorescent molecules were used as fluorescence probes for the study of bio-mimetic micro-heterogeneous environments [18], and for sensing the local polarity of the microenvironment around their binding sites on biologically relevant systems like proteins [16]. Fluorescent probe spectroscopy is also rapidly gaining importance as a non-invasive efficient technique compared to the complex and

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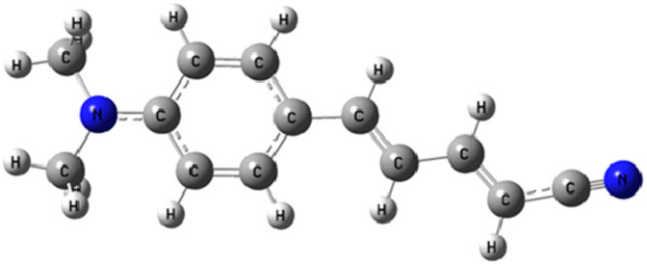

Scheme 1. Optimized structure of 5-(4-dimethylamino-phenyl)-penta-2,4-dienenitrile (DMAPPDN) at HF/6-31+G(*d*,*p*) level. The final structure was generated by Gauss View software.

expensive techniques such as X-ray or NMR analysis for studying the chemical unfolding of these proteins induced by agents like guanidine hydrochloride, urea and surfactants. In recent times, the studies of structural and dynamical aspects of such biological systems using new synthetic extrinsic polarity sensitive fluorescent probes are gaining extra momentum especially when the experimental findings corroborate the results of molecular docking [19–21] and molecular dynamics simulation [19,22,23].

In this work, we report the use of a specially designed polarity sensitive intramolecular charge transfer molecule 5-(4-dimethylamino-phenyl)-penta-2,4-dienenitrile (DMAPPDN) (Scheme 1) as an extrinsic fluorescent probe for studying BSA microenvironment [24]. Monitoring denaturation with guanidine hydrochloride was also attempted using the spectral response of the probe. Steady state absorption, emission, fluorescence anisotropy, REES, CD and time resolved spectral measurements have been used in this context. Molecular level interactions, conformational changes of protein BSA after binding with the probe and flexibility at the binding sites have been explored by spectroscopic measurement hand in hand with molecular docking and molecular dynamics simulations. This work is very unique because our designed molecule, DMAPPDN can probe protein structure and dynamics easily with only absorption and emission spectral responses and molecular modeling without involving any complex X-ray or NMR analysis. Also this study makes way for the probable application of this specially designed molecule DMAPPDN as an effective fluorescent probe for spectroscopic investigation of such other biological systems.

2. Experimental methods

2.1. Reagents

The synthetic scheme, procedure and purification of DMAPPDN have been described in our earlier publication [24]. GdnHCl and BSA were purchased from SRL India and used as received. 10^{-3} M DMAPPDN, 10^{-6} M BSA and 9 M GdnHCl solutions were prepared in 0.01 M Tris–HCl buffer solution corrected to pH = 7.4 by the addition of 1:1 HCl and used as stock solutions. Triply distilled water was used for preparing all solutions. The purity of all solvents in the studied wavelength range was checked before the preparation of solutions. All solutions of DMAPPDN and BSA were prepared at the desired concentrations and equilibrated for 6–7 h before spectral measurements.

2.2. Measurement of steady state absorption and emission and CD spectroscopy

All steady-state absorption spectra were recorded on a Hitachi UV/VIS U-3501 spectrophotometer. The emission spectra and fluorescence anisotropy were recorded on a Perkin Elmer LS-55 fluorescence spectrophotometer after proper background corrections with individual solvents. Concentration of the probe was kept at $\sim 10^{-6}$ M for all measurements to ensure no occurrence of self aggregation or self quenching and also to maintain probe concentrations at a much lower value than the protein. The alterations in the secondary structure of the protein in the presence of probe were studied by far UV circular dichroism measured by a Jasco Corporation, J-815 CD spectrophotometer using a quartz cuvette of path length 0.1 cm at 1 nm data pitch intervals. All CD spectra were recorded in the wavelength range 190–250 nm. The spectrophotometer was sufficiently purged with 99.9% nitrogen before measurements. The spectra were collected at a scan speed of 50 nm/min with response time of 1 s at 298 K temperature. Each spectrum was baseline corrected with Tris–HCl buffer and the final plot was taken as an average of four accumulated plots.

2.3. Time resolved spectral measurement

Fluorescence lifetimes were determined from time-resolved intensity decay by the method of time correlated single-photon counting (TCSPC) using a picosecond diode laser (IBH, U.K. nanoLED) [25] as the light source at 370 nm. The typical instrumental response of this excitation source is ~40 ps. A Hamamatsu MCP photomultiplier tube (5000U-09) collected the emission at a magic angle polarization. The TCSPC setup consists of an Ortec 9327 CFD and a Tennelec TC 863TAC. Data collection was done with a PCA3 card (Oxford). An IBM DAS6 software was used to deconvolute the fluorescence decays.

2.4. Molecular docking study

Molecular docking was performed to obtain the protein-ligand binding energy and to identify the potential ligand binding sites. The docking experiments were performed with the help of AutoDock4.2 [26] and AutoDock Tools (ADT) software using the Lamarkian Genetic Algorithm (LGA) based on the adaptive local method search. The energy based Autodock scoring function includes terms accounting for short range van der Waals and electrostatic interactions, loss of entropy upon ligand binding, hydrogen bonding and solvation. For the recognization of the binding sites in BSA, docking was carried out with setting of grid box size 126 Å × 96 Å × 126 Å along *x*, *y*, *z* axes covering whole protein with a grid spacing 0.508 Å [19] after assigning the protein and probe with Kollman charges. The grid center was set at 0.026, 0.108, and 0.114 Å. At first, AutoGrid was run to generate the grid map of various atoms of the ligand and receptor. After the completion of grid map, ligand flexible docking simulations were performed with 200 runs and 2.5×10^6 energy evaluations, 27,000 numbers of generations, 50 GA population and root mean square cluster tolerance 2.0 Å [23] per run. Among 200 runs 10 minimum energy conformers were chosen according to ranking and scoring [20,23]. Finally the lowest energy conformation was used for docking analysis.

2.5. MD simulation protocol

The MD simulations were carried out using NAMD 2.6 software [27] and CHARMM22 force field and parameters [28]. Preparation of protein and probe structures has been mentioned in the supplementary data. Protein structure file (psf) for both the BSA and probe of our interest were prepared by Vega ZZ 2.4 (http://www.vegazz.net) [29] software. At first, the protein was neutralized with 17 Na^+ ions after being immersed in TIP3P water box containing 25,372 water molecules with box dimension of 95 Å × 86 Å × 98 Å by using VMD 1.8.7. software [30]. We have prepared two water boxes, first one containing water, protein and Na^+ ions with total numbers of 85,251 atoms and the other box with water, protein, Na^+ ions and the probe with 85,280 atoms.