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Study of microheterogeneous environment of protein Human Serum Albumin by an extrinsic fluorescent reporter: A spectroscopic study in combination with Molecular Docking and Molecular Dynamics Simulation

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ABSTRACT

We report extrinsic fluorescent probe 5-(4-dimethylamino-phenyl)-penta-2,4-dienoic acid (DMAPPDA) as a molecular reporter for studying microheterogeneous environment of protein Human Serum Albumin (HSA) via spectral modification of the probe under physiological condition. Steady state emission, fluorescence anisotropy, Red Edge Excitation Shift (REES), far-UV Circular Dichroism (CD), Atomic Force Microscopy (AFM) imaging, time resolved spectral measurements, Molecular Docking and Molecular Dynamics (MD) Simulation techniques have been used to fulfill this achievement. Interaction of the probe with HSA is signaled by the blue shift of the fluorophore emission maxima with enhancement of fluorescence intensity. The increase in steady state anisotropy, REES and fluorescence lifetime values with increasing protein concentrations indicates interaction and movement of the probe from free aqueous media to the more restricted less polar hydrophobic interior of protein. Experimental results obtained from Benesi–Hildebrand plot support the formation of 1:1 HSA–DMAPPDA complex with high binding constant and negative free energy change. Thermal denaturation of the probe bound protein has also been tracked using the spectral response of DMAPPDA. Molecular Docking studies revealed binding of the probe with in the hydrophobic cavity of subdomain IIA of HSA. MD Simulation supports greater stability of HSA–DMAPPDA complex compared to free protein.

1. Introduction

For years, in the field of photochemistry and photobiology, intramolecular charge transfer (ICT) fluorescent probes containing donor and acceptor groups are finding new avenues for multidirectional applications such as pH and ion detectors, for creation of new optoelectronic devices such as electroluminescence devices, solar cells and thin film transistors [1–4], chemical sensors [5] for free volume measurement in polymers and degree of water penetration into the surfactant aggregates, probes for the study of bio-mimetic environments [6] for sensing the local polarity around the binding sites of biologically relevant systems like proteins [7–9]. In this context, fluorescent probe spectroscopy [10] is also rapidly gaining importance as a non-destructive yet efficient and highly sensitive technique for studying of proteins as well as their chemical and thermal unfolding processes. In recent times, the studies on structural and dynamical aspects of biological systems like proteins, enzymes, etc. using new synthetic extrinsic polarity sensitive fluorescent probe are gaining extra momentum especially when the experimental results find support from theoretical studies like Molecular Docking [11–14] and MD Simulation [13,15,16].

Human Serum Albumin (HSA) is the most abundant protein (0.6 mmol dm^{-1}) in the blood plasma and acts as a transport protein [17,18] and is capable of binding, delivering an extraordinarily diverse range of endogenous and exogenous compounds like fatty acids, nutrients, steroids, certain metal ions, hormones, enzymes, surfactants and a variety of therapeutic drugs [19,20] through the bloodstream to their target organs [21]. The crystal structure of HSA is well established [22]. The primary structure of HSA is a sequence of 585 amino acid residues and the secondary structure contains 67% alpha helix with six turns and 17 disulfide linkages [11,12]. The tertiary structure is formed by three homologous domains I → III, each of which is divided into two subdomains A and B [22,23]. HSA has only one tryptophan residue Trp-214 in the IIA subdomain [11,16]. This protein also contains two principal drug binding sites, Site-I and Site-II. Site-I is located with in the hydrophobic cavity of subdomain IIA which is capable of binding mostly neutral, bulky heterocyclic compounds by strong hydrophobic interactions, whereas Site-II is with in the IIIA subdomain and binds many aromatic carboxylic acids by dipole–dipole, van der Waals and hydrogen bonding interactions [24]. Although a large volume of research is already reported in diverse fields with

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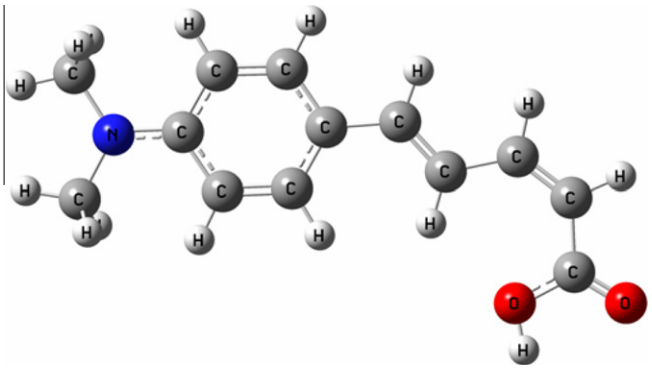
HSA as a model protein, but the intrigue regarding the various types of hydrophobic, hydrophilic and electrostatic interactions within the protein interior in presence and absence of small molecules like drugs and/or fluorescent probe [25], etc. remains vibrant even today. Various researchers have studied the structure and properties of HSA and their interactions with other proteins, drugs, ligands and with denaturants such as guanidine hydrochloride, urea and surfactants [7,26–30] using NMR [31–33], dynamic light scattering, differential scanning calorimetry [34,35], CD [12,15] and other techniques.

In the present work, we report the use of our specially designed polarity sensitive intramolecular charge transfer molecule 5-(4-dimethylamino-phenyl)-penta-2,4-dienoic acid (DMAPPDA) (Scheme 1) [36] as an extrinsic fluorescent reporter for studying HSA microenvironment. It is reported that donor–acceptor aromatic charge transfer molecules have shown a crucial role in biological light harvesting processes such as photosynthesis [37]. We have chosen this charge transfer molecule keeping in mind that the derivative of this type of molecules with an extra acceptor group attached have been used as conjugated organic dyes for efficient dye sensitized solar cells in recent times [38]. Main interest is to study location and nature of binding of small extrinsic reporter molecule with HSA. Monitoring of thermal denaturation of the probe bound protein was also attempted using the spectral response of the probe. Steady state absorption, emission, fluorescence anisotropy, REES, CD, AFM imaging and time resolved spectral measurements have also been used in this context. Molecular level interactions, conformational changes of the protein HSA after binding to the probe and the flexibility at the binding sites have been explored by the experimental measurement techniques along with Molecular Docking and Molecular Dynamics Simulations. This study makes way for the probable application of this specially designed donor–acceptor charge transfer molecule DMAPPDA as an effective fluorescent probe for spectroscopic investigation of a model biological system.

2. Materials and methods

2.1. Chemicals

DMAPPDA was synthesized and purified according to the procedure described in our earlier publication [36]. Fatty acid and globulin free Human Serum Albumin (⩾99%) was purchased from Sigma–Aldrich Chemicals Pvt. Ltd. and used as received. 10^{-6} M HSA solutions was prepared in 0.01 M Tris buffer corrected to pH = 7.4 by addition of 1:1 HCl and used as stock solution. Since the probe is not totally soluble in Tris–HCl buffer, 1: 20 MeOH: Tris–HCl buffer solution was used to prepare 10^{-3} M stock DMAPPDA solution. The purity of all solvents in the studied wavelength range was checked before preparation of solutions. All solutions of DMAPPDA and HSA were prepared at the required concentrations and equilibrated for 6–7 h before spectral measurements.



Scheme 1. Optimized structure of 5-(4-dimethylamino-phenyl)-penta-2,4-dienoic acid (DMAPPDA) at HF/6-311++G(*d*,*p*) level. The final structure was generated by Gauss View software.

2.2. Measurement of steady state emission, anisotropy, REES and CD spectroscopy

All steady-state emission spectra were recorded on a Perkin Elmer LS-55 fluorescence spectrophotometer equipped with a quartz cell of path length 5 mm and a thermostat bath 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 that of protein. In all the emission spectra and anisotropy measurement, the probe molecule was excited at 345 nm with the excitation and emission slits keeping 5 nm. In case of fluorescence measurement for FRET experiment, the donor HSA was excited at 295 nm and emission spectra were recorded 305–580 nm wavelength range. For REES experiment emission spectra were recorded by 20 nm red shifting of the excitation wavelength (345–365 nm). Alteration of secondary structure of the protein in the presence of probe were studied by far UV CD spectra, 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. Atomic Force Microscopy

To observe the morphology of the free HSA, probe DMAPPDA and HSA–DMAPPDA complex, 90 μM HSA, 5 μM DMAPPDA and equilibrated 90 μM HSA + 5 μM DMAPPDA solution drop were placed on mica foil, dried in vacuum and kept over night for taking AFM image. The samples were imaged in tapping mode operating at resonate frequency of 267.87 KHz using VEECO multimode nanoscope-IIIa system (USA). RTESPA silicon tip was used for all experiments.

2.4. Measurement of fluorescence lifetime

Fluorescence lifetimes were measured from time-resolved intensity decay by the method of time correlated single-photon counting (TCSPC) using a picosecond diode laser (IBH, UK nanoLED) [39] as the light source at 340 nm. The typical instrument response of this excitation source is ~80 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.

2.5. Molecular Docking Simulations

Molecular Docking was performed to obtain the protein–ligand binding energy and to identify the potential ligand binding sites. The blind docking experiments were performed with the help of AutoDock4.2 [40] and AutoDock Tools (ADTs) software using the Lamarkian Genetic Algorithm (LGA) based on the adaptive local method search. The energy based AutoDock scoring function in-