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Deciphering the fluorescence resonance energy transfer from denatured transport protein to anthracene 1,5 disulphonate in reverse micellar environment

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ABSTRACT

Constrained environmental effect inside AOT reverse micellar media has been employed in this work to collect the information about energy transfer efficacy between sodium salt of anthracene 1,5 disulphonate (1,5-AS) with model transport proteins, bovine serum albumin (BSA), and human serum albumin (HSA). Steady state, time-resolved fluorescence and circular dichroism techniques have been used for this purpose and corresponding Förster-type resonance energy transfer (FRET) from tryptophan residues to 1,5-AS indicates that 1,5-AS binds in the vicinity of the tryptophan residue (BSA and HSA) with equal strength. Indication of protein damage from fluorescence data and its confirmation has been measured from CD measurement. Molecular modeling study hereby plays a crucial role to predict the minimum energy docked conformation of the probe inside the protein environment. From the docked conformation the distance between 1,5-AS and tryptophan moiety of BSA/HSA has successfully explained the FRET possibility between them. A comparative modeling study between BSA and HSA with 1,5-AS assigning their binding site within specific amino acids plays a crucial role in support of the FRET study.

1. Introduction

In applied fields, spectroscopic techniques for probing the structure, dynamics, and function of the biological system mainly based on fluorescence from the tryptophan residue buried within the core of the protein molecules have been raised its interest now a day's [1,2]. Serum albumins, rich in plasma, are the most widely studied proteins. Structural aspects and properties of these transport proteins have been well explored. These transport proteins of 580 amino acid residues are composed of a single polypeptide chain and are characterized by a high content of cystine, stabilizing a series of nine loops and a low content of tryptophan. These serum albumins consist of 67% of helix of six turns and 17 disulfide bridges in their secondary structures [3]. The tertiary structure is composed of three α-helical domains I-III. Each domain consists of two subdomains named as IA, IB, IIA, IIB, IIIA, and IIIB [4]. Bovine and human serum albumins (BSA and HSA respectively) exhibit approximately 80% ordered homology and a repeating pattern of strictly conserved disulfide. The high affinity photosensitizer-binding sites on serum albumin have been classified into two well-exemplified groups, sites I and II, which are located in hydrophobic cavities in subdomains IIA and IIIA. As domain II and III share a common interface, binding a probe to domain III leads to conformational changes affecting the binding affinities to domain II. Despite the size and complexities of HSA, there is only a single tryptophan residue (Trp-214) in domain II which facilitates the study of protein from a spectroscopic point of view. In the case of BSA, there are two tryptophan residues (Trp 134 and Trp 212). Trp-212 is found localized in a similar hydrophobic microenvironment as single tryptophan residue of HSA but additional tryptophan residue (Trp-134) in BSA is found localized in the second helix of the first domain and is more exposed to solvent [5,6].

Energy transfer phenomena have wide applications in energy conversion processes. One consequence of energy transfer is photosensitization. One important example of the latter is photosynthesis [7]. Photodynamic action, which is often used in the treatment of cancer, is also a consequence of energy transfer [7]. Excited-state energy transfer to dye mixtures has been used to achieve better dye laser performance at a specific wavelength. Fluorescence study of RET has also been used to study protein folding and subunit interactions in media containing confined

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water. Spectroscopic method has extensively been applied for investigation of drug binding with albumins under physiological conditions because of its accuracy, sensitivity, rapidity, and convenience in handling. Fluorescence resonance energy transfer (FRET) is a fluorescence phenomenon during which energy is transferred non radiatively from one fluorescent molecule (donor) to a second fluorophore (acceptor) [8]. It has been widely used to study the structure and dynamics of molecules in the gas phase, solution phase, and solid state [8,9]. Fluorescence resonance energy transfer is a powerful spectroscopic technique that allows biological relevant distances between 20 and 80 Å to be quantified under physiological conditions with near angstrom resolution due to the strong distance dependence of the transfer process. According to Förster, the excitation spectrum of the acceptor must overlap with the emission spectrum of the donor [10,11]. The radiative orientation of the transition dipoles of the participant also has an influence on the efficiency of energy transfer, and the FRET process is strongly dependent on the distance between the participants. According to Förster's theory the rate of energy transfer depends mainly upon the following factors [10,12–14]: (a) the extent of spectral overlap between the donor emission and the acceptor absorption spectra, (b) the quantum yield of the donor (ϕ_f), (c) the relative orientation of the donor and acceptor transition dipoles, and (d) the distance between the donor and acceptor transition dipoles.

The photophysics and photochemistry of various anthracene sulphonate (AS) have been widely used in protein separation and refolding in the downstream processing of biotechnology [15,16]. In this context, we, hereby investigate the FRET characteristics of 1,5-AS and protein in AOT/n-heptane RM medium. The importance of organized assemblies such as micelles, reverse micelles (RM) which are used as membrane biomimetic agents, lies in their capacity to provide a matrix for efficient interaction [17–19]. Keeping in mind the versatile uses of energy transfer phenomena, this work has been carried out to investigate the energy transfer prospect in correlation with protein structure in AOT/n-heptane reverse micellar medium. As reverse micelles act as a model bio mimicking system due to its constrained water pool in w/o system, FRET application inside this water pool with transport proteins and a fluorophore certainly a primary step to the next bimolecular research.

2. Materials and method

2.1. Materials

Sodium salt of anthracene 1,5 di-sulphonate (1,5-AS) was synthesized by reducing the corresponding anthraquinone with Zn dust and 20% NaOH solution for 4–6 h [20] BSA (98% fraction V SRL), HEPES buffer(N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulphonicacid]), HSA(Sigma, >96%), AOT (Aldrich) and *n*-heptane were used as received. The concentration of 1,5-AS used in RM solution was of the order of 10^{-6} (M). All experiments were done at same ω value (ω = 20) at 298 K.

2.2. Methods

The absorption and emission spectra were recorded on Shimadzu (model UV1700) UV–vis spectrophotometer and Shimadzu spectrofluorimeter (model RF 5301) respectively. Fluorescence decay curves were obtained from time resolved intensity decay by the method of time-correlated single photon counting (TCSPC) using a nanosecond diode LED at 280 nm (IBH, nanoLED) as a light source. The data stored in a multichannel analyzer was routinely transferred to IBH DAS-6 decay analysis software. Circular dichroism (CD) was measured by a Jasco spectropolarimeter (J-815), equipped with a Jasco Peltier-type temperature controller using a rectangular quartz cuvette of path length 1 cm. The measurements were carried out at 298 K. Far UV-CD spectra were collected with a protein concentration of 0.18 μM with 1.0 cm path length in the range of 200–240 nm. For only CD measurement HEPES buffer of 1 mM was used.

The crystal structure of HSA (PDB entry 1AO6), BSA (PDB entry 4F5S) were downloaded from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). AutoDock Vina (with standard settings) was used to perform the molecular docking [21]. MGL tools (version 1.5.4) was used to prepare the ligand (1,5-AS) and receptor (protein) for docking and the graphical front end for setting up and running the AutoDock docking software. The 3D structure of the 1,5-AS was obtained from Sybyl 6.92 (Tripos Inc., St. Louis, USA) and the energy-minimized structure was achieved using a Tripos force field and Gasteiger– Hückel charges with a gradient of 0.005 kcal/mol. The defined grid box covered the entire computative binding site and the lowest energy configuration was considered the best docking pose for 1,5-AS. The output from AutoDock is rendered with PyMOL [22] which has also been used to calculate the distances between nearest atoms which interact with each other.

3. Results and discussion

3.1. Steady state behavior of 1,5-AS

The UV–Vis absorption spectra of 1,5-AS was recorded in AOT reverse micellar (RM) environment (ω = 20) having λ_{max}^{abs} at 366 nm and corresponding absorption spectrum is inserted in Fig. 1. The absorption band in this region corresponds to л → л* transition (first excited singlet, S_1) of the molecule. The absorption spectra of 1,5-AS in RM media was remain unchanged upon individual addition of both BSA and HSA. So the possibility of ground state complex formation between 1,5-AS and the serum albumins inside the reverse micellar core is ruled out.

In RM media the fluorescence spectrum of 1,5-AS was increased in intensity (Fig. 2a) with maximum at 414 nm on selective excitation at 366 nm with addition of both BSA and HSA. To obtain an insight into the binding interaction between 1,5-AS and serum albumins, the binding constant values have been estimated from the fluorescence intensity data using Almgren equation [23].

$$\frac{F_\infty - F_0}{F - F_0} = 1 + \frac{1}{K[protein]} \quad (1)$$

where F_0, F and F_∞ are the fluorescence intensities of 1,5-AS in absence of protein, in presence of intermediate protein concentration and at a protein concentration when the interaction is almost complete, respectively. K represents the binding constant and [protein] is the total protein concentration. Plots of $(F_\infty - F_0)/(F - F_0)$ versus $[protein]^{-1}$ in relation to Eq. (1) for both BSA and HSA show linear variations (Fig. 2b) in RM environment. The binding constant values were calculated from the slope of the individual plots and are given in Table 1. For BSA and HSA, in buffer media the binding constant values are 8.7×10^4 and 24.4×10^4 mol^{-1} dm^3 respectively [24] whereas in the RM environment, for BSA and HSA the values are 2.5×10^5 and 2.4×10^5 mol^{-1} dm^3 respectively. From binding constant values it is evident that in RM environment, probe binds strongly with protein than buffer media. It is evident that the binding constant of probe, 1,5-AS with BSA and HSA is identical in RM environment. This is probably due to the equal accessibility of Trp of both proteins to 1,5-AS.