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Correlation of FRET efficiency with conformational changes of proteins in ionic and nonionic surfactant environment

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ABSTRACT

We have studied the effect of interaction of surfactants on the FRET process in aqueous solution from transport proteins, BSA and HSA to the probe molecule, sodium salt of anthracene 1,5-disulphonate (1,5-AS). Cationic surfactant CTAB and gemini, anionic surfactant SDS and nonionic surfactant igepal-630 have been used for our investigation as surfactants and micellar solution. In organized media denaturation of protein occurs. Denaturation of protein has also been observed in presence of 1,5-AS. This has been established from steady state, time resolved fluorescence and circular dichroism (CD) studies in homogeneous and heterogeneous environments. Helecity calculations from CD spectra have also supported the results. Nonionic and ionic micellar solutions have been found to directly affect the protein helicity and the FRET parameters. The FRET parameters and denaturation of proteins in homogeneous and heterogeneous media have been compared.

1. Introduction

Serum albumins, rich in plasma, are the most widely studied proteins and serve as a protein storage component. Despite the size and complexities of human serum albumin (HSA), there is only a single tryptophan residue (Trp-214) whereas in bovine serum albumin (BSA), there are two tryptophan residues (Trp 134 and Trp 212) [1]. BSA and HSA are frequently used in biophysical and biochemical studies and have many physiological functions, including the ability to bind different categories of small molecule, drug and other bioactive molecules because of the availability of hydrophobic pockets inside the protein network and the flexibility of the albumins in adapting their shapes [2,3]. For the study of dynamics of biological molecules, anthracene and its derivatives (here sodium salt of anthracene-1,5-disulfonate i.e. 1,5-AS) are used as fluorescent probes for their high fluorescence yield [4]. On sulfonation, the bi-sulfonate becomes water-soluble and produces interesting fluorescence characteristics. The sulfonate group affects the overall symmetry of the anthracene moiety and also introduces a negatively charged centre in the molecule in aqueous solution. The probe is widely used in drug delivery, chemiluminescence [5,6], protein separation and refolding in the downstream processing of biotechnology [7,8].

The importance of organized assemblies such as micelles and reverse micelles which are used as membrane biomimetic agents, lies in their capacity to provide a matrix for efficient interaction [9,10]. Gemini surfactant ($C_nC_mC_n$) consists of two hydrophobic chains, two polar head groups, and a spacer linked at or near the head groups. These are superior to the conventional single-chain surfactants in many properties such as low Kraft temperature, low critical micelle concentration (CMC), and strong hydrophobic microdomain [11,12] and are known as 'second generation surfactants'. There are many studies regarding the interaction of proteins in different systems but the behavior of the protein molecules and their interaction with small molecules in micellar systems both in pre-micellar and micellar region of ionic and nonionic surfactants is rare in literature. Here, we have studied the spectral characteristics of 1,5-AS in three different kinds of surfactant solutions: sodium dodecylsulphate (anionic: SDS), cetyltrimethyl ammonium bromide (cationic: CTAB), gemini, (cationic: $C_{14}C_5C_{14}$ or G_5), and igepal co-630 (nonionic: Ig-630). A photo physical process i.e. fluorescence resonance energy transfer (FRET) can gauge protein folding and interactions in media containing confined water in biochemical events. Due to FRET, simultaneous quenching of donor fluorescence and enhancement of acceptor fluorescence can take place [13]. FRET in various confined geometries, such as micelles, vesicles, clays, and zeolite, is specifically interesting [14]. In particular, FRET in micelles has been widely investigated not only due to the biological significance associated with the similarity between the micelles and biological membranes but also for the possible use of FRET as probes for micelle studies and biomedical imaging. In this study, we report efficient FRET between albumin and probe molecule (1,5-AS) in HEPES buffer medium as well as in different surfactant media.

The aim of this work is to investigate the extent of conformational change of serum albumins that occurs upon binding with probe in HEPES buffer, and at different concentrations of surfactant solution (i.e. below and above CMC of each surfactant). The feasibility of energy transfer and the comparison of energy transfer efficiency in different media have also been determined. These reveal details about the

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structural heterogeneity of proteins, and the structural fluctuations of these molecules over moderately large distances (~44 Å). The efficiency of energy transfer depends on the distance between donor and acceptor. This phenomenon can be used to determine the depths of chromophore in proteins relative to the macromolecule–water interface [13].

The choice of the fluorescent probe molecule is important because its residence depth in the interface of micelles is used to estimate the polarity and nature of the region in which it is located. The absorbance and fluorescence characteristics of 1,5-AS in aqueous and different micellar and pre-micellar media have been studied to characterize the role of micelles on the photo physics of 1,5-AS. Time resolved fluorescence spectra were taken for confirmation of FRET and circular dichroism (CD) was studied to investigate the secondary structure of both albumin proteins.

2. Materials and methods

Materials and methods are described in supplementary data.

3. Results and discussion

3.1. Emission behavior of 1,5-AS in presence of protein

The emission spectra of 1,5-AS in the presence of different concentrations of protein were recorded on excitations of the probe at 366 nm (λ_{exc}) in HEPES buffer (10 mM, pH = 7) as well as in pre-micellar and micellar environments of ionic (CTAB, SDS, G_5) and non-ionic (Ig-630) surfactants. In the absence of protein an emission band of 1,5-AS at 414 nm in all the above environments was obtained. There is no change in emission spectra of 1,5-AS with addition of protein in pre-micellar and micellar media of SDS (Fig. S1). However on addition of proteins (BSA/HSA), the emission intensity of 1,5-AS decreases in other cases. The decrease of fluorescence intensity of 1,5-AS in presence of protein can be rationalized in terms of binding of the probe (1,5-AS) with protein. The binding constants between the probe and the protein have been determined from the fluorescence intensity data following the method described by Almgren et al. [15]. According to this method

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

where F_0, F, and F_∞ are the fluorescence intensities of 1,5-AS in the absence of protein, in protein solution, and under conditions of complete saturation of binding between probe and protein, respectively. K represents the binding constant between the probe in the excited state and protein. Apart from aqueous buffer medium, the binding parameters between probe and protein moiety were also studied in cationic (CTAB, G_5) and nonionic surfactant (Ig-630) environment. The plot of $(F_\infty - F_0) / (F - F_0)$ vs. 1 / [protein] (Eq. (1)) shows the linearity (Fig. 1) in all experimental media. The binding constant values in different media are tabulated in Table 1. For BSA, in pre-micellar region of CTAB, G_5 and Ig-630 the binding constant values are 69.3×10^4, 66.3×10^4 and 8.2×10^4 mol^{-1} dm^3 respectively whereas in micellar region of CTAB, G_5 and Ig-630 the values are 6.1×10^4, 7.4×10^4 and 10.0×10^4 mol^{-1} dm^3 respectively and in HEPES buffer media the value is 8.7×10^4 mol^{-1} dm^3. From binding constant values (Table 1) it is evident that in nonionic micellar solution of Ig-630, probe binds strongly with protein than pre-micellar solution whereas for cationic surfactants (CTAB and G_5) in pre-micellar solution, surprisingly, probe binds strongly with protein compared to micellar solution. This difference may be due to the presence of stern layer in cationic micelles and palisade layer in nonionic micelles. The absence of change in emission

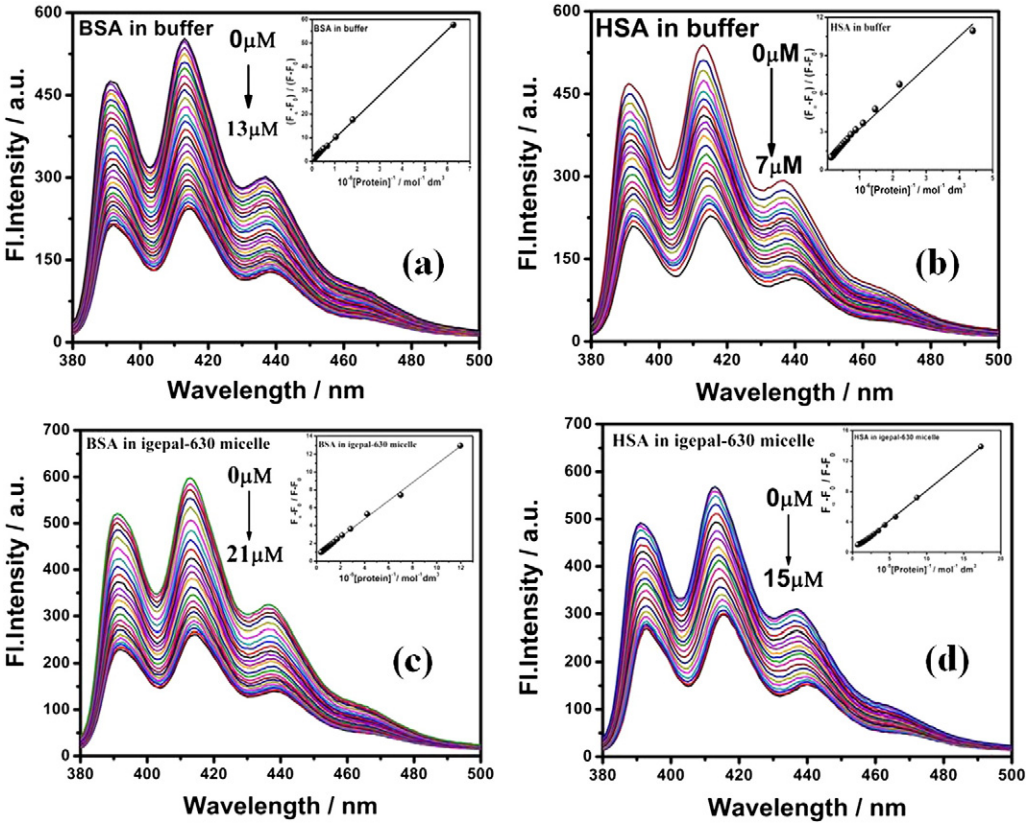

Fig. 1. Fluorescence spectra of 1,5-AS solution with increasing BSA concentrations (λ_{exc} = 366 nm) in (a) HEPES buffer medium (b) micellar solution of Ig-630 medium; fluorescence spectra of 1,5-AS solution with increasing HSA concentrations (λ_{exc} = 366 nm) in (c) HEPES buffer medium (d) micellar solution of Ig-630 medium. The plot of $(F_\infty - F_0)/(F - F_0)$ vs. $[\text{protein}]^{-1}$ are given on the inset in each cases.