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Role of hydrophobic and polar interactions for BSA–amphiphile composites

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ABSTRACT

To evaluate the role of hydrophobic and electrostatic or other polar interactions for protein–ligand binding, we have studied the interactions of bovine serum albumin (BSA) with 2-alkylmalonic acid and 2-alkylbenzimidazole amphiphiles having different head group and alkyl chain length. The binding affinity for the protein–amphiphile interactions is found to depend predominantly on the length of hydrocarbon chain, suggesting the crucial role of hydrophobic forces, supported by polar interactions at the protein surface. The BSA fluorescence exhibits appreciable hypsochromic shift along with a reduction in fluorescence intensity and mean lifetime upon binding with 2-alkylmalonic acid. UV–visible, steady state and time-resolved fluorescence measurements were performed to compare the effects of amphiphiles on BSA as a function of the amphiphiles head group and alkyl chain length.

1. Introduction

The marginal stability of the native globular conformation of proteins, which is a delicate balance of various interactions in the proteins, is affected by the pH, temperature and the addition of small molecules such as substrates, coenzymes, inhibitors and activators that bind specifically to the native state. Studies on the interactions of surfactants with globular proteins can contribute towards an understanding of the action of surfactants as denaturants and as solubilizing agents for membranes of proteins and lipids. Extensive studies on the interactions of surfactants with globular proteins have been reported and reviewed (Jones and Brass, 1991). The interaction of proteins with amphiphiles/surfactants has received a great deal of interest for many years due to its application in a great variety of industrial, biological, and cosmetics systems (Dickinson, 1993; Jones, 1992; Turro et al., 1995; Takeda et al., 2002). Surfactants can be broadly classified into those which bind and initiate protein unfolding, i.e. denaturing surfactants and those that only bind leaving the tertiary structure of the protein intact. Commonly ionic surfactants such as sodium *n*-dodecyl sulphate generally denature proteins whereas non-ionic surfactants do not. There are, however, exceptions to this rule too (Jones et al., 1987; El-Sayert and Roberts, 1985). The fluorescence properties of the tryptophan residues in folded proteins vary widely (Eftink, 2000, 1994; Kronman and Holmes, 1971). The quantum yields vary from near 0 to 0.35, and the wavelength where the quantum yield is maximal, λ_{max}, varies from as low as 308 nm for buried tryptophan residues to near 350 nm for tryptophan residues that are largely exposed to solvent (Alston et al., 2004; Callis and Liu, 2004; Pan et al., 2006).

A number of studies have focused on the multifunctional binding properties of bovine serum albumin (BSA) (Peters, 1985, 1988; Foster, 1977; Chakraborty et al., 2009) which binds a wide variety of molecules. The binding function is a means of transporting soluble substances between tissues and organs. Binding also functions as a protection against the toxic effects of the bound ligand. Binding studies with BSA find broad and significant applications in the area of rational drug design as many pharmaceuticals are rendered less effective or entirely ineffective by virtue of their interaction with BSA. BSA functions biologically as a carrier for fatty acid anions and other simple amphiphiles in a blood stream. It has a molecular weight of 66,411 g mol^{-1} and contains 583 amino acids in a single polypeptide chain. The protein contains 17 disulphide bridges and one free –SH group, which can cause it to form a covalently linked dimer. The protein is known to have a heart-shaped structure (N form) in solution with a net charge of −18 on its surface. The interior of the protein is almost hydrophobic, while both the charged amino acid residues and the apolar patches cover the interface (Vijai and Forster, 1967; Curry et al., 1998). Based on our previous work of amphiphile–protein interactions (Ojha and Das, 2010), here we have reported, the effects of hydrocarbon chain length and nature of the hydrophilic group on the photophysical response of BSA, using two different types of amphiphilic molecules viz. 2-alkylmalonic acid (amphiphile **1**) and 2-alkylbenzimidazole (amphiphile **2**) having different lengths of aliphatic chain (Scheme 1). In this work, we have employed UV–visible, steady-state and time-resolved fluores-

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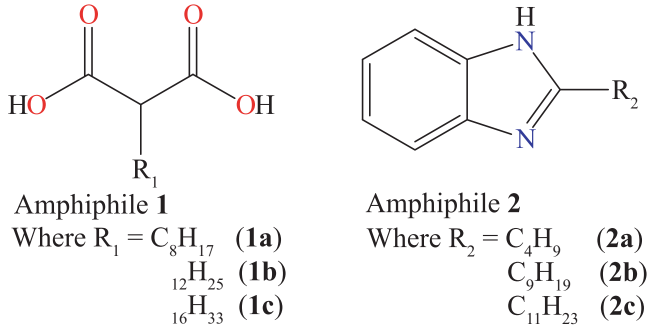

Scheme 1. Structures of the amphiphiles **1** and **2**.

cence measurements in order to obtain information related to the binding mechanism of the probes to BSA such as binding modes, binding constants, binding sites and quenching rate constants as a function of the amphiphiles head group and alkyl chain length. The results may cast some light on the future study of interaction between amphiphiles and other proteins.

2. Experimental

2.1. Materials

Commercially available diethyl malonate, *o*-phenylenediamine, aliphatic acids and alkyl halide were obtained from Merck, India and Sigma, USA. BSA was purchased from Fluka Germany and was used as supplied. Other chemicals were of reagent grade and used without further purification. A stock solution of BSA (10 μM) in 10 mM aqueous phosphate buffer of pH 7.0 was prepared, while the amphiphiles stock solution were prepared in DMSO because of their lower solubility in water. For interaction studies of amphiphiles with protein, a 3.0 mL aqueous solution of BSA (10 μM) was titrated with various concentrations of the amphiphiles ranging from 0 to 24 μM, where the total volume of DMSO did not exceed 15%. The presence of 15% DMSO induces no major BSA structural changes (Supporting information). Each solution was mixed thoroughly before spectral measurements at room temperature.

2.2. Methods

The absorption spectra were recorded on a Perkin Elmer Lambda-25 UV–visible spectrophotometer using 10 mm path length quartz cuvettes in the range of 200–350 nm wavelengths, while fluorescence measurements were taken on a Carry eclipse spectrofluorometer using 10 mm path length quartz cuvettes with slit width of 5 nm by exciting the protein solution at 295 nm. An excitation wavelength of 295 nm was applied to selectively excite the tryptophan residues in protein. Background intensities of the buffer blanks in which BSA was omitted were subtracted from each sample spectrum to cancel out any contribution due to the solvent. Time-resolved intensity decays of the protein were measured using a Life Spec II spectrofluorimeter (Edinburgh instrument) at 298 K. The sample was excited by Pico-quant 290 nm light emitting diode (LED) and the decay was measured through a 50 ns time scale at a time resolution of 0.0122 ns/channel. The decay curves were analyzed by FAST software using discrete exponential method, provided by Edinburgh instrument. The generated curves for intensity decay were fitted in the functions of Eq. (1), where, α_i is the initial intensity of the decay component i, having a lifetime τ_i, (Swaminathan et al., 1994).

$$I(t) = \sum \alpha_i \exp\left(\frac{t}{\tau_i}\right) \qquad (1)$$

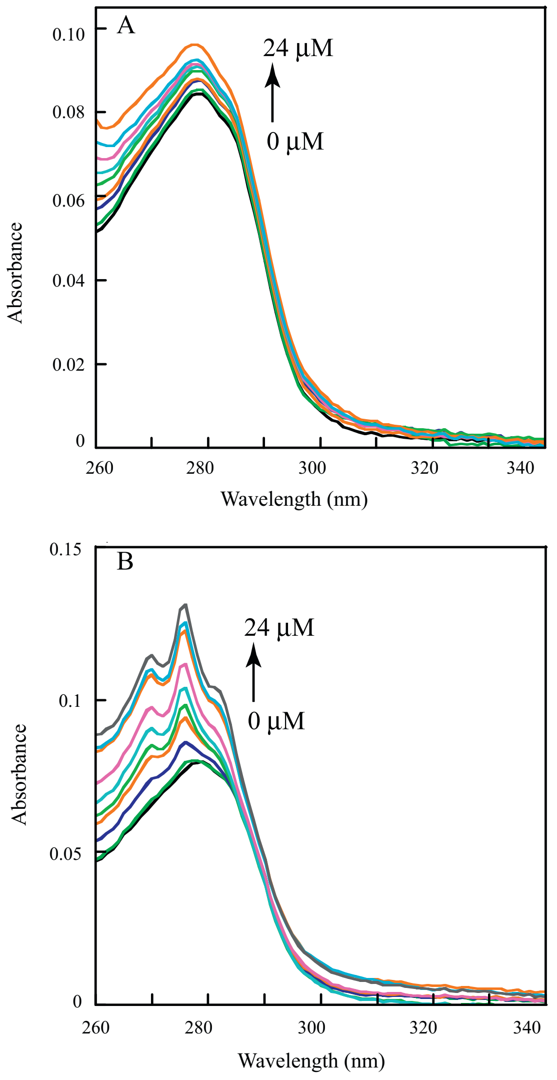

Fig. 1. UV–visible absorption spectra of BSA (3.0 μM) with increasing concentrations of (A) amphiphile **1a** and (B) amphiphile **2a** from 0 to 24 μM in phosphate buffer of pH 7.0.

2.3. Synthesis of amphiphiles

2-Alkylmalonic acid (amphiphile **1**) and 2-alkylbenzimidazloes (amphiphile **2**) were prepared by the following literature methods (Pool et al., 1937; Hui et al., 2007; Palou et al., 2005; Aggett and Timperley, 1969; Nokami et al., 1979). The structures of the amphiphiles were shown in Scheme 1. The purity of the compounds were checked by NMR, IR, melting point and were found to be in good agreement with the reported data (Supporting information).

3. Results and discussion

3.1. UV–visible spectra of BSA in the presence of the amphiphiles

UV–visible absorption spectrum is a very simple and applicable method to explore the structural change and to know the complex formation in solution (Hu et al., 2004). Fig. 1 shows the UV–visible absorption spectra of BSA in the presence of different concentrations of the amphiphiles (**1** and **2**). Amphiphile **1** has no absorption under the presence experiment conditions. As can be seen from Fig. 1, free BSA has maxima at 279 nm and it increases with the addition of the amphiphiles (**1** and **2**), while free amphiphile **2** has two bands at 280 and 274 nm, showed gain in intensity upon com-