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A study on the interaction of horse heart cytochrome *c* with some conventional and ionic liquid surfactants probed by ultraviolet-visible and fluorescence spectroscopic techniques

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ABSTRACT

The interactions of a protein cytochrome *c* with some selected conventional and ionic liquid surfactants have been investigated at pH 7.4 using ultraviolet-visible and fluorescence spectroscopic techniques. We used four conventional surfactants - cetyltrimethylammonium bromide (CTAB), dodecyltrimethylammonium bromide (DTAB), sodium *N*-dodecanoylsarcosinate (SDDS), and *N*-decanoyl-*N*-methylglucamine (Mega 10), and a surface active ionic liquid 1-hexadecyl-3-methylimidazolium chloride (C_{16}MeImCl). All the investigated surfactants were found to induce an unfolding of the protein cytochrome *c*. In presence of CTAB, SDDS and C_{16}MeImCl, the heme iron atom was found to loose methionine from its axial position. Differential binding of the surfactant monomers and their micelles to the protein molecules was inferred. The ionic surfactants were found to be more effective than the nonionic one in unfolding the investigated protein. However, the extent of binding of CTAB/C_{16}MeImCl to cytochrome *c* reaches a plateau past the critical micellization concentration (*cmc*) of the surfactant. For each of the cytochrome *c*-DTAB, cytochrome *c*-SDDS and cytochrome *c*-Mega 10 system, although there exists an inflection in the surfactant-binding, saturation point could not be detected. It has been demonstrated from the ultraviolet-visible spectral studies that the oxidation state of iron in cytochrome *c* does not change when the protein binds with the investigated surfactants.

1. Introduction

Solutions comprising of polyelectrolytes and surfactants exhibit a rich and intricate aggregation behavior [1–10]. Such a behavior owes its origin to the various types of interactions between the polyelectrolytes and surfactants when simultaneously present in a solution. These interactions have been attributed to the subtle interplay of electrostatic forces between the surfactant molecules and the polyion, and hydrophobic forces between the hydrocarbon tails of amphiphile ions. Because of the widespread domestic, industrial, and technological applications of polyelectrolyte-surfactant mixtures [2,5,6], for example, in detergency, cosmetics, and drug delivery, enhanced oil recovery, paints, pharmaceuticals, biomedical application, food and mineral processing, considerable attention has been paid to these systems.

The interactions of proteins with surfactants in aqueous solutions have been the subject of intense research because of the potential applications of protein-surfactant formulations in pharmaceuticals, detergents, cosmetics, paints, coatings, bioelectronics and biochemical reactions [11–14]. Surfactants generally perturb the three dimensional structure of proteins, and, therefore, studies on protein-surfactant interactions can provide important information on the conformational alterations of proteins [15]. Well-characterized proteins are usually selected to study their interaction with surfactants [14,16–20]. Cytochrome *c* is one of the best characterized proteins in terms of structure, both in the crystalline state [21] and in solution [22–24]. It is a globular heme protein consisting of a single polypeptide chain of 104 amino acid residues. It serves as an essential link in the electron transport chain through which cells perform the controlled “burning” of glucose and capture much of the released energy by storing it in ATP, the cell's primary energy distribution molecule. Due to the strong field histidine and methionine coordination at axial positions, the low spin state of cytochrome *c* is stabilised [17,21,25,26]. In horse heart cytochrome *c*, the heme group is bonded to four amino acids and, is also connected to a noncovalent side-chain, thus making the internal structure around the heme macrocycle relatively rigid. Because of the formation of the covalent thiol bondings with the heme group, the proximal histidine bond is not generally cleaved under denaturing conditions. Thus, cytochrome *c* serves as a very good model for investigating the unfolding-refolding phenomena of the polypeptide chain where the heme group participates simultaneously in the process without bimolecular recombination [27–34]. Despite the fact that studies on protein-surfactant interactions could provide important clue to protein unfolding processes, very few reports on the interaction of cytochrome

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c with conventional surfactants [35–40], and only one report on cytochrome *c* interactions with an ionic liquid surfactant [17] in aqueous media exist thus far in the literature.

Here, we have made an attempt to unravel the nature of interactions of cytochrome *c* with a number of selected conventional and ionic liquid surfactants in aqueous media at physiological pH using ultraviolet-visible and fluorescence spectroscopic techniques.

Five surfactants namely, cetyltrimethylammonium bromide (CTAB), dodecyltrimethylammonium bromide (DTAB), sodium *N*-dodecanoyl sarcosinate (SDDS), *N*-decanoyl-*N*-methylglucamine (Mega 10), and 1-hexadecyl-3-methylimidazolium chloride (C_{16}MeImCl) have been selected for the present study. DTAB and CTAB are single chain cationic surfactants. SDDS is an amino-acid based anionic surfactant which is a salt of long chain N-acyl amino acids. It is biodegradable and it has also antimicrobial activities [41,42] Mega 10 is a sugar-based nonionic surfactant with a hydrophilic sugar moiety and a hydrophobic alkanoyl chain [43]. Mega 10 is also biodegradable and it finds application in food, cosmetics and cleaning products [16]. C_{16}MeImCl is an imidazolium-based surface active ionic liquid (SAIL). The structures of the surfactants are given in Table 1. A comparison of the conformational alterations in cytochrome *c*, if any, induced by these surfactants particularly in the pre- and post-micellar regimes will be made.

2. Materials and Methods

2.1. Materials

Horse heart cytochrome *c*, DTAB (purity: 99%), SDDS (purity: 99%), Mega 10 (purity: 99%) and C_{16}MeImCl (purity: 98%) were purchased from Sigma-Aldrich whereas CTAB (purity: 99%) was obtained from Alfa Aesar (UK). Sodium dihydrogen phosphate and sodium phosphate were purchased from S. D. Fine-Chem Pvt. Ltd. and Loba Chemie respectively. All these chemicals used as received.

2.2. Preparation of Buffer Solution

The phosphate buffer solution of pH 7.4 has been prepared by mixing sodium dihydrogen phosphate and sodium phosphate solutions using the standard protocol. Then, all the protein and surfactant solutions are prepared in this phosphate buffer medium.

2.3. Ultraviolet-visible Absorption Studies

Absorption spectra of the cytochrome *c* solution were measured in a UV 1601 Shimadzu (Japan) spectrophotometer using 10 mm path length quartz cuvette. The spectra were recorded in 250–600 nm wavelength range. 2.5 ml 2 μM cytochrome *c* solution in phosphate buffer of pH 7.4 was taken in the cuvette and then concentrated surfactant solution was added in a stepwise manner and continued to a concentration well above its *cmc*.

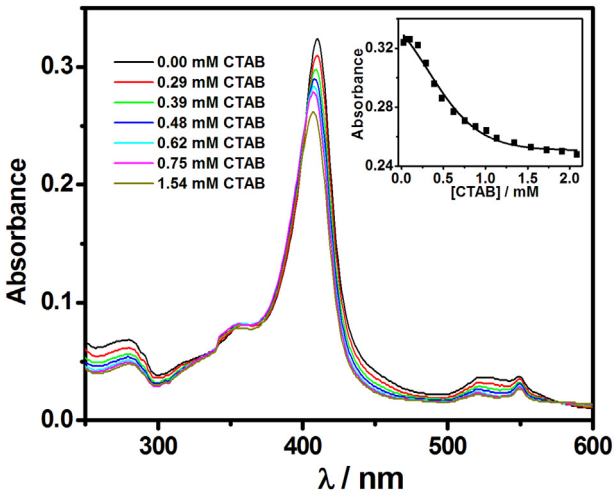


Fig. 1. Absorption spectra of 2 μM cytochrome *c* solution in absence and in the presence of increasing concentration of CTAB (*cmc* 0.8 mM) in phosphate buffer at a pH of 7.4. Inset shows absorbance vs. [CTAB] plot at 410 nm wavelength.

2.4. Fluorescence Emission Studies

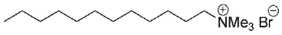
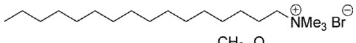
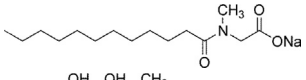
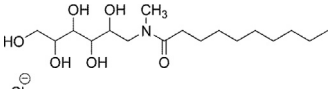
The fluorescence emission spectra and intensity have been recorded on a Perkin Elmer LS 55 fluorescence spectrometer using a 10 mm path length quartz cuvette. Fluorescence spectra have been measured from 310 to 500 nm with excitation and emission slit widths fixed both at 15 nm. The excitation wavelength is fixed at 280 nm. 2.5 ml 2 μM cytochrome *c* solution in phosphate buffer at a pH of 7.4 is taken in the cuvette; then concentrated surfactant solution is added in a stepwise manner from a very low concentration to a concentration well above the *cmc*. The emission spectra are recorded after each excitation. The scan rate has been fixed at 250 nm per minute.

3. Results and Discussion

3.1. Ultraviolet-visible Absorption Spectra

The ultraviolet-visible spectrum of cytochrome *c* in phosphate buffer medium of pH 7.4 shows a number of absorption bands (Fig. 1). A band at 280 nm arises due to the n-π* transition of aromatic amino acids tryptophan, tyrosine and phenylalanine. An intense $S_0 \rightarrow S_2$, Soret band observed at 409 nm is assigned to π-π* transition of the heme group wherein the iron atom is imposed upon by axial histidine and methionine ligands. Further, the weak $S_0 \rightarrow S_1$ Q bands at 528 nm and

Table 1
Molecular structures of the surfactants.

Surfactant	Molecular structure
DTAB	
CTAB	
SDDS	
Mega 10	
C_{16}MeImCl