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Effect of Furosemide on denaturation of lysozyme in the presence of ionic surfactant at different temperatures

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ABSTRACT

The thermodynamics of Furosemide–lysozyme interaction has been studied using conductivity in combination with viscosity, density and speed of sound measurements. Lysozyme binds preferentially with Furosemide in the presence of surfactants, sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB). A number of physico-chemical parameters viz. critical micelle concentration (CMC), standard enthalpy of micellization (ΔH_m°), standard entropy of micellization (ΔS_m°), standard free energy of micellization (ΔG_m°), apparent molar volume (ϕ_v), apparent molar adiabatic compressibility (ϕ_k) and relative viscosity (η_r) have been calculated from experimentally measured conductivity (κ), density (d), speed of sound (v) and viscosity (η). The critical micelle concentration data were found to pass through a broad minimum at 303 K, which tends to become sharp with the increase in the [lysozyme] in case of SDS. Whereas for CTAB, CMC values, in general increase with the temperature and decrease with the increase in concentration of lysozyme. Experiments at different lysozyme concentrations and in the presence of surfactants suggest the predominant involvement of electrostatic interactions in the complexation process of Furosemide with lysozyme in the presence of SDS which induce unfolding of lysozyme as the surfactant concentration approaches its critical micelle concentration value, and non-specific interaction behaviour of Furosemide with lysozyme in the presence of CTAB.

1. Introduction

Drug binding to protein is considered as the interaction between a biological macromolecule and a small molecule. Studies on such systems provide valuable fundamental information regarding the mechanism by which drug exerts its pharmacologic and therapeutic effects [1–9]. Biological reactions are driven by interaction of a host of different molecules with a number of receptors. Most of such receptors are protein molecules whose proper function is vital for an orderly life process. Enzymes are often target molecules for different drugs and in nature they occur, in multiple forms with different physico-chemical properties [10,11]. Almost every drug exerts its pharmacologic effect by interacting with some protein in the body and is eliminated from the body principally by combining with some kind of transport system or drug-metabolizing enzyme.

Protein–surfactant interactions have been extensively studied in aqueous solutions [12–22]. The binding of surfactant molecules to proteins occurs by a combination of ionic and hydrophobic interaction. The studies suggest that protein–surfactant interactions are significantly affected at concentrations well below the CMC of surfactant. The studies include both similarly charged and oppositely charged protein–surfactant systems [23–27].

Various surfactants have been utilized in their ability to enhance the permeability of drugs across biological membranes. The interaction of the drug with surfactants revolves around physico-chemical interactions between drug molecules and surfactants in solution. These physico-chemical interactions manifest themselves in terms of enhanced solubility and dissolution of the drug, prevention of drug precipitation if administered in solution form and reduction in drug activity. Knowledge of the mechanisms of interaction between drugs and proteins is of crucial importance in understanding the pharmacodynamics and pharmacokinetics of a drug [28]. Therefore studies on drug–protein interactions have increasingly attracted the research attention of chemists, pharmacists and biologists [29].

Lysozyme is an antimicrobial enzyme. It is one of the smallest water soluble globular proteins consisting of 129 amino acid residues, out of which 18 are cationic and 12 are anionic having molecular weight of 14,350 g mol^{-1}. Its structure is stabilized by four disulphide bridges [30]. The interior of the protein is almost entirely hydrophobic, whereas the surface is covered both by the charged amino acid residues and non-polar regions. The

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Table 1
CMC, 10^3 and corresponding X_{CMC}, 10^4 (CMC in mole fraction) values of SDS and CTAB in aqueous solutions of 0.001 M Furosemide containing different concentrations of lysozyme at different temperatures.

T (K)	Lysozyme (%)							
	CMC, 10^3				X_{CMC}, 10^4			
	0	0.10	0.25	0.50	0	0.10	0.25	0.50
SDS								
293	5.8	8.4	9.1	9.45	1.04	1.51	1.64	1.70
298	6.2	7.4	8.4	9.00	1.12	1.33	1.51	1.62
303	6.5	7.1	8.4	9.00	1.18	1.28	1.51	1.62
308	7.0	7.7	9.4	10.00	1.26	1.39	1.69	1.80
313	7.5	8.8	11.0	11.80	1.35	1.58	1.98	2.12
CTAB								
293	5.3	4.50	3.95	3.80	0.95	0.810	0.711	0.684
298	5.6	4.70	4.20	4.00	1.01	0.846	0.756	0.720
303	5.9	4.90	4.38	4.20	1.06	0.882	0.788	0.756
308	6.2	5.14	4.60	4.40	1.12	0.925	0.828	0.792
313	6.5	5.35	4.80	4.60	1.18	0.963	0.864	0.828

isoelectronic point of lysozyme is at pH 11.0 and therefore positively charged in aqueous solution. The importance of lysozyme relies on its extensive use as a model system to understand the underlying principles of protein structure, function, dynamics and folding, through theoretical and experimental studies [31,32]. High natural abundance is also one of the reasons for choosing lysozyme as a model protein for studying protein–surfactant interaction.

In this paper, we focus on the study of opposite as well as similar charged protein–surfactant systems, namely lysozyme-sodium dodecyl sulphate and lysozyme-cetyltrimethyl ammonium bromide in the presence of drug Furosemide. These systems feature several interesting aggregation phenomena and can be used as model systems. The results presented in these studies concern the main forces involved in the formation of aggregates in protein–surfactant systems, by concentrating on two relevant stages, the protein–surfactant complex formation and the surfactant micellization.

2. Experimental

2.1. Materials and methods

SDS and CTAB of AR grade were obtained from SD Fine Chem. Ltd., and were purified as described in the literature [27,33]. Lysozyme from chicken egg white was obtained from HiMedia. It was, however stored at ~4–5 °C, and used without giving any additional treatment. Furosemide was provided by Aventis Pharma. Ltd. Mumbai. Distilled deionized water of specific conductance, κ value ~$1–3 \times 10^{-6}$ S cm^{-1} was used in all preparations. Conductivity measurements were carried out using a Cyberscan Con-510. The measured conductances were accurate within ±0.5%. Stock solutions of SDS and CTAB of molar concentrations ranging from 1 to 18 mM for SDS and 0.6 to 15 mM for CTAB were gradually added to the 14 ml of 0.001 M Furosemide containing 0.1, 0.25 and 0.5% lysozyme solution maintained at a constant temperature. The conductance values were recorded for each addition after thorough mixing and temperature equilibration. The temperature was kept constant using a calibrated automatic digital temperature controlled high precision water thermostat supplied by Narang Scientific Works (NSW) – New Delhi with precision of ±0.1 °C and circulated through the conductivity cell using a high powered digital water circulator supplied by Riviera Pvt. Ltd. Mumbai. The critical micelle concentration (cmc) was obtained from the change in the slope of the measured conductance versus surfactant concentration plot. Density and speed of sound of SDS solution were measured from high precision Anton Paar DSA-5000. The temperature was maintained to ±0.001 °C. The reproducibility of speed of sound and density data was ±0.5 m s^{-1} and $\pm 5 \times 10^{-6}$ kg m^{-3} respectively. Viscosity measurements were carried out with a jacketed Ostwald viscometer. The precision achieved in viscosity measurement was ±0.01%.

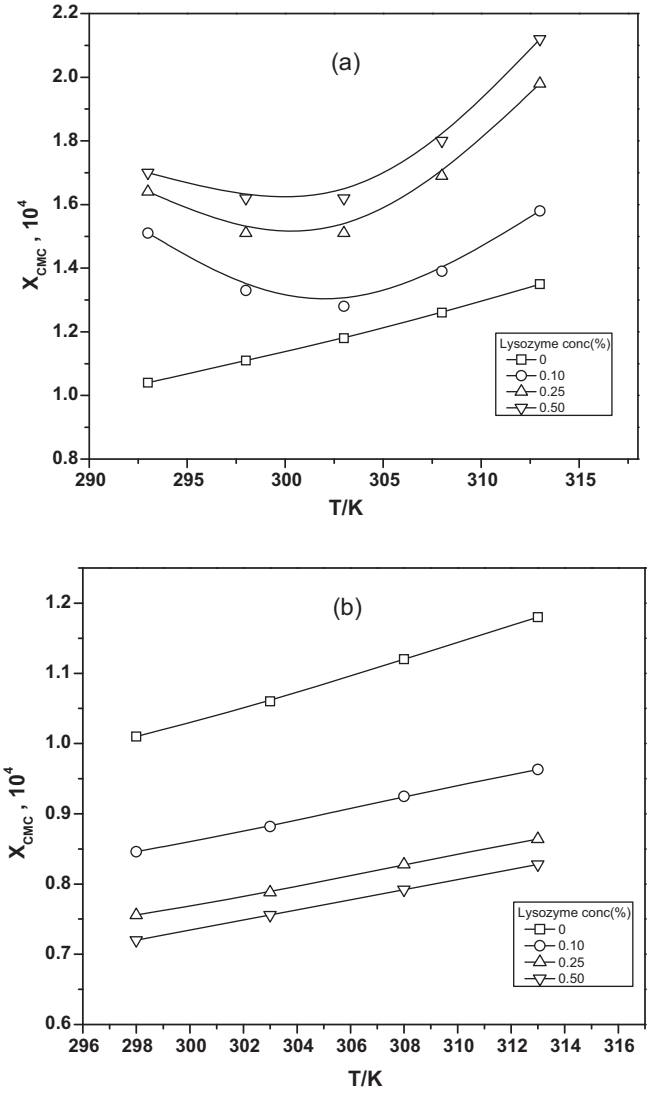

Fig. 1. Plot of X_{CMC} vs. temperature of (a) SDS and (b) CTAB in aqueous solution of 0.001 M Furosemide containing different concentrations of lysozyme.