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Interfacial and thermodynamic approach of surfactants with α-chymotrypsin and trypsin: A comparative study

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Abstract
The conductivity as well as surface tension measurements have carried out to study the interactions among cetyltrimethyl ammonium bromide (CTAB), sodium dodecyl sulpahe (SDS) with α-chymotrypsin (α-CT) and trypsin in aqueous medium on pH 7.75. The surface parameter *i.e.,* critical micelle concentrations (CMC), the maximum surface excess concentration (Γ_{max}), minimum area per surfactant molecule (A_{min}) and the surface pressure at CMC (π_{cmc}), and thermodynamic parameters *i.e.,* degree of ionization (α), Gibbs free energy of micellization (ΔG°_{m}), the standard Gibbs energy of adsorption (ΔG°_{ads}), the free energy at air-water interface (ΔG^{s}_{mim}) have been evaluated using surface tension and conductivity measurement. Thermodynamic parameters indicate that enzyme-CTAB/SDS monomeric aggregation started to form micelles at a higher concentration of surfactant to compare with the CMC of pure CTAB/SDS micelles.

Keywords: α-chymotrypsin, trypsin, surfactant, micellization behavior, conductivity, surface tension

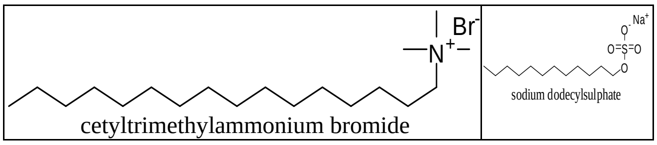
Introduction

Surfactant molecules are shows the aggregation behaviors of various molecules i.e., DNA, Enzyme, ionic liquids, amino acid & enzyme under different condition [1, 4]. Surfactant is surface active agent and consist of hydrophilic and hydrophobic group [5]. Hydrophilic part is water soluble and hydrophobic is water fearing [6]. Surfactants have been show the different properties, *i.e.* high detergency, high viscoelasticity, high surface wetting capability, high solubilization [7], a better tendency to lower the oil–water interfacial tension than their single chain analogues [8, 9]. Surfactant has been shown various application field *i.e.,* wetting agents, cleaning agents, dispersants, foaming agents, emulsifiers, soaps and shampoos, antiseptics, corrosion inhibitors [10].

Trypsin and α-Chymotrypsin (α-CT) are significant mechanism of the enzymatic barrier [11]. They can mortify the beneficial proteins and peptides, inhibit their activity as a result, and thus decrease their oral bioavailability [12]. As individual kind of indirect protease inhibitors, have shown proof of concept in clinical trials [13].

The surface-active molecules into micellar aggregates are mentioned to as micellization [14]. Surfactants has been varied intensive properties in the solution such as 'self-assembly', this called micelles, and the concentration of development occurs is denoted the critical micelle concentration (CMC) [15, 17]. Varma *et al*. [18] has been studied the interactions among cetyltriphenylphos-phonium bromide (CTPB) with α-chymotrypsin (α-CT) and trypsin in aqueous medium at pH 7.75. The surface parameter and thermodynamic parameters calculated using surface tension and conductivity method. The hydrolysis of p-nitrophenyl acetate (PNPA) and p-nitrophenyl benzoate (PNPB) catalyzed by trypsin in the presence of CTPB, CTAB and SB3-12.

In present investigation, to study the interactions among cetyltrimethyl ammonium bromide (CTAB), sodium dodecyl sulpahe (SDS) with α-chymotrypsin (α-CT) and trypsin in aqueous medium on pH 7.75. Critical micelle concentrations (CMC), surface parameter and thermodynamic parameter have been evaluated using surface tension and conductivity methods. The chemical structure of cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS) are shown in Scheme 1.



Experimental Section

Materials

Cationic surfactants such as cetyltrimethylammonium bromide (CTAB) sodium dodecyl sulfate (SDS), potassium chloride (KCl), α-chymotrypsin (α-CT) and trypsin were purchased from Sigma Aldrich Pvt. Ltd. Banglore india with high purity. All the aqueous solutions were prepared using double distilled water.

Methods

Surface tension measurements were obtained using a Jencon tensiometer. The specific conductance (κ) studies were carried using a digital conductivity meter (Systronics Type-306).

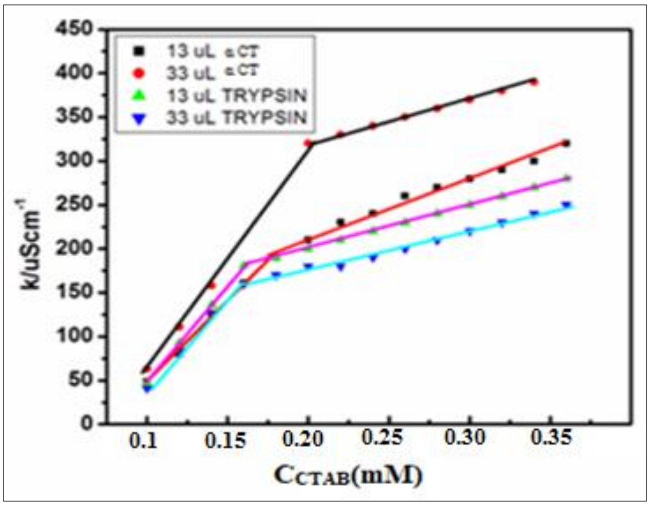


Fig 1: Specific conductance (κ) vs concentration of CTAB (mM) in the presence of different concentration of α-CT and Trypsin at 300K.

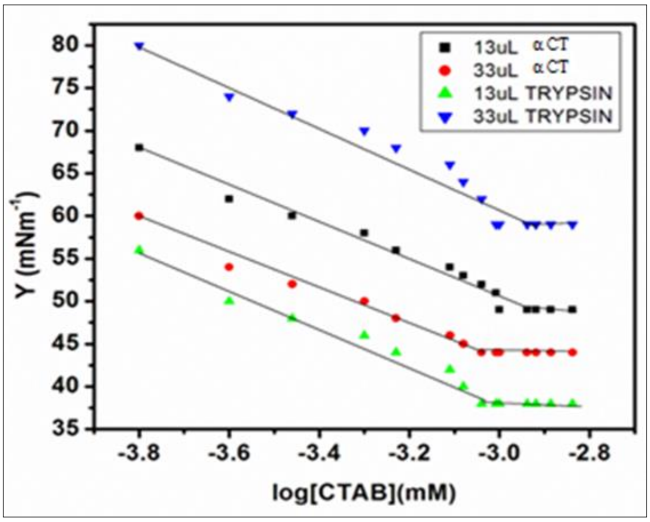


Fig 2: Plots of surface tension vs. log CTAB concentration in water at different concentration of α- CT / Trypsin.

Results and discussion

(A) Critical Micelle Concentration

The critical micelle concentration (CMC) for cationic i.e., cetyltrimethylammonium bromide (CTAB) and anionic i.e., sodium dodecyl sulfate (SDS) surfactants with enzyme (CTAB+ trypsin/α-CT and SDS+ trypsin/α-CT) was determined with the help of conductivity method at 300K. The graph plot between specific conductivity (κ) with total concentration of surfactant solution is shown in a fig. 1

Table 1: Critical micelle concentration of cetyltrimethylammonium bromide (CTAB) sodium dodecyl sulfate (SDS) in the presence of α-CT/ Trypsin media at 300 K.

Types of Proteins	Volume of Protein (uL)	CMC (mM)			
		Cond.	S. T.	Cond.	S. T.
		CTAB		SDS	
α-CT	water	1.2	1.0	8.1	8.1
	13	2.0	2.0	10.0	11.0
	33	2.6	2.4	13.8	13.5
Trypsin	water	1.2	1.0	8.1	8.1
	13	1.8	1.6	12.2	12.8
	33	2.4	2.2	14.5	14.2

The degree of micellar ionization (α) was calculated the slope ratio of pre-micelle/post-micelle ($\alpha = C_{Pre\text{-}micelle}/C_{Post\text{-}micelle}$). Table-1, shows the CMC value of all CTAB/SDS and mixture of α-CT, Trypsin surface tension and conductivity technique have been used to calculated CMC value in good agreement with reported in the literature value [7]. The CTAB+Trypsin/α-CT and SDS+Trypsin/ α-CT interaction was measured by tensiometric method as shown in fig. 2 and 3. A lineally decrease in surface tention (γ) was observed with increase in CTAB/SDS concentration for all the systems. The anionic surfactants are more interaction in both enzyme (trypsin/ α-CT) because of the presents in anions (SO_4^{-2}) is a most responsive to the hydrophobic interaction except CTAB.

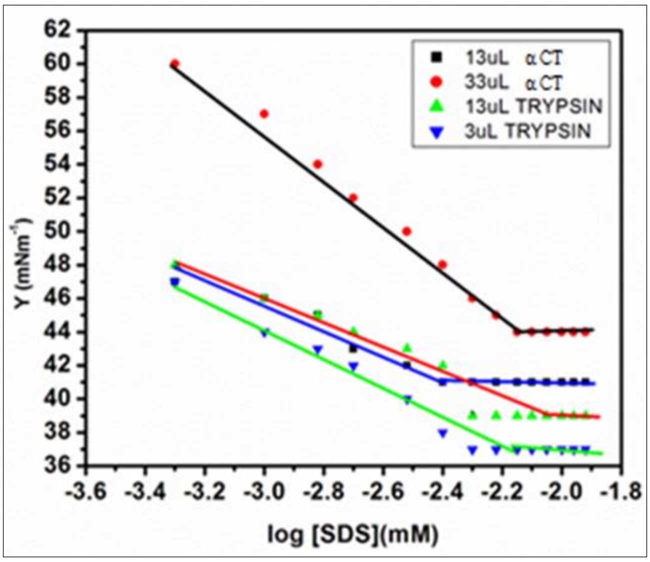


Fig 3: Plots of surface tension vs. log SDS concentration in water at different concentration of α- CT /Trypsin.

(B) Interfacial properties of cationic and anionic surfactants in enzyme

Cationic and anionic surfactants consist at the air/water/solution interface as well as air/protein solution interface and decrease surface tension (γ) of water or protein solution. The surface parameters were calculated by literature reported by Ghosh *et al.* [19] and Banjare *et al.* [20]. The interfacial adsorption per unit area of surface at various concentration of CTAB/SDS surfactant can be calculated with the help of Gibbs adsorption Equation. The maximum surface excess ($\acute{\Gamma}_{max}$), at CMC has been evaluated the following Gibbs adsorption equation (1).

$$\Gamma_{max} = -\frac{1}{2.303nRT}\left[\frac{d\gamma}{d \log C}\right]_{T,P} \quad (1)$$

$$A_{min} = 1/\Gamma_{max} N_A \quad (2)$$

$$\pi_{CMC} = \gamma_o - \gamma_{CMC} \quad (3)$$

Where R, T and C are gas constants, temperature and concentration respectively. The constant 'n' (pre-factor) is 2. At the air/water interface, the minimum are of the per surfactant molecule (A_{min}) Equation (2) and the surface pressure at the CMC (π_{cmc}) equation (3) value are presented in Table-2. Its observed the $\acute{\Gamma}_{max}$ value are increased with increases the concentration of the enzyme (Trypsin/α-CT) in the mixture, Table-2, shown the $\acute{\Gamma}_{max}$ value are higher for SDS except CTAB. The values of Γ_{max} decreases where, A_{min} increases due to reduction of forces between the head group of surfactants with enzyme.

π_{CMC} values depend on the interfacial area occupied by cationic/anionic surfactants with their precise position and the structure at the interface [21]. The decrease of the γ_{cmc} of the aqueous solution by the suspension of the surfactants indicates more effective adsorption at the interface of enzyme [22]. Increasing the concentration of enzyme decreases the π_{CMC}, because of the goods of surfactants.

(B) Thermodynamic properties of cationic and anionic surfactants in enzyme

Trypsin/ α-CT are modified thermodynamic properties of both CTAB and SDS surfactants. The various types of intermolecular forces are involved for the micellization behavior of surfactants such as van der Waals forces [23], dipole-dipole interaction and hydrogen bonding are involved for the interaction of trypsin/ α-CT with CTAB/SDS surfactants. The standard Gibbs free energy of micellization (ΔG°_{m}), Gibbs free energy of micellization per alkyl tail (ΔG°_{m},tail), Gibbs energy of transfer (ΔG_{trans}), standard free energy of adsorption (ΔG_{ads}) and Gibbs free energy of the given air/water interface (G^{s}min) of Gemini surfactants were calculated by using following Equation (4) to (8);

$$\Delta G^{0}_{m} = (2 - \alpha)\, RT \ln X_{CMC} = (2-\alpha) \ln \frac{C_{CMC}}{55.4} \quad (4)$$

$$\Delta G^{\circ}_{ads} = \Delta G^{\circ}_{m} - \pi_{CMC} / \Gamma_{max} \quad (5)$$

$$\Delta G^{(s)}_{min} = A_{min} \cdot \gamma_{CMC} \cdot N_{A} \quad (6)$$

$$\Delta G^{\circ}_{m;tail} = \frac{\Delta G^{\circ}_{m}}{2} \quad (7)$$

$$\Delta G^{\circ}_{trans} = \Delta G^{\circ}_{m\,(solvent\ mixed\ media)} - \Delta G^{\circ}_{m\,(pure\ water)} \quad (8)$$

where, α is the counter ion dissociation, R is the ideal gas constant, T is the temperature in Kelvin and X_{CMC} is the CMC in mole fraction unit. The calculated ΔG°_{m} values become more negative as the trypsin/ α-CT concentration increases, which is due to stronger hydrophobic interactions among CTAB/SDS. The calculated value of ΔG°_{m} is listed in Table 2. The negative values of ΔG°_{m} leads to the spontaneous process in all system. The standard free energy of micellization (ΔG^{O}_{m}), standard free energy of adsorption (ΔG^{O}_{ads}) in the CTAB/SDS interaction with trypsin/ α-CT is presented in the Table 2. ΔG^{O}_{ads} and ΔG^{O}_{m} are negative in all case and become more negative as the trypsin/ α-CT concentration increase inducting that aggregates become more favorable at higher trypsin/ α-CT content. The calculated $\Delta G^{(s)}_{min}$ values are concerned with the free energy change of the solution components from the bulk phase to the surface phase of the solution [24]. Table 2, are indicates the lower $\Delta G^{(s)}_{min}$ values specify a more thermodynamically stable surface in CTAB/SDS micellar solution in the presence of trypsin/ α-CT.

The CTAB/SDS surfactants tail transfers Gibbs free energy from trypsin/ α-CT mixture to hydrobhobic core of micelle. The tail part of surfactant apart owing to solvophobic effects. As compared with the pure medium, the additions of trypsin/ α-CT compose more favorable for CTAB/SDS surfactant molecule and the hydrophobic group to move from the bulky phase into micellar phase. As a result, Table 2 shows the ΔG°_{trans} increases with the increase in the CMC values of CTAB/SDS surfactants system.

Table 2: Surface excess parameter (Γ_{max}), surface pressure at CMC (π_{CMC}), minimum surface area per molecule (A_{min}), degree of ionization (α), Gibbs free energy of micellization (ΔG°_{m}), the standard Gibbs energy of adsorption (ΔG°_{ads}), the free energy at air-water interface (ΔG^{s}_{mim}), Gibbs free energy of micellization per alkyl tail (ΔG°_{m},tail), Gibbs energy of transfer (ΔG_{trans}) for cationic (CTAB) and anionic (SDS) surfactant in the presence of α-CT/ Trypsin media at 300 K.

Types of Proteins	Volume of Protein (uL)	Interfacial Prameter			Thermodynamic Parameter					
		Γ_{max} (10^{4} mol·m^{-2})	A_{min} 10^{20} (m^{2} mol^{-1})	π_{cmc} (mN·m^{-1})	ΔG^{s}_{min} kJ/mol	ΔG^{o}_{m} kJ/mol	ΔG^{o}_{ads} kJ/mol	α	ΔG°_{m},tail kJ /mol	ΔG_{trans} kJ /mol
CTAB										
α-CT	water	1.89	87.5	34.0	2.17	-15.60	-29.46	0.44	-7.8	-
	13	0.89	67.2	26.0	0.23	-11.56	-28.94	0.90	-5.78	4.04
	33	1.26	47.7	38.0	0.27	-7.43	-74.37	0.72	-3.71	8.47
Trypsin	water	1.89	87.5	34.0	2.17	-15.60	-29.46	0.44	-7.8	-
	13	0.76	78.6	27.0	0.28	-11.10	-29.60	1.98	-5.55	4.5
	33	0.94	63.7	37.0	0.34	-8.23	-43.49	0.58	-4.11	7.37
SDS										
α-CT	water	1.19	1.39	39.0	2.76	-61.38	-94.05	0.66	-30.84	-
	13	1.03	16.05	33.0	37.72	-8.66	-43.56	1.18	-4.33	52.72
	33	7.76	21.37	31.5	53.69	-4.58	-45.83	0.74	-2.29	56.8
Trypsin	water	1.19	1.39	39.0	2.76	-61.38	-94.05	0.66	-30.84	-
	13	1.19	72.27	28	0.36	-8.01	-18.06	1.18	-4.00	53.38
	33	7.14	43.04	31	0.57	-5.56	-35.79	0.74	-2.78	55.82

Conclusion

The micellization behaviours of cationic and anionic surfactants in trypsin/ α-CT have been systematically determined using conductivity and surface tension techniques.

1. CMC values decrease with increasing the V/V % of DESs in solution.
2. N_{agg} in the micelle increases with increasing V/V% of

trypsin/ α-CT because of the change in the interfacial Gibbs energy.
3. The negative values of ΔG°_{m} and ΔG°_{ads} show that the micelle formation and adsorption of amphiphiles at the air/water interface is energetically favorable. Various thermodynamic parameters of micellization show that increasing V/V % of trypsin/ α-CT make micellization more favorable.
4. Low value of ΔG^{s}_{min} ensures the stability of mixed micelles.

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