Activity of α-Chymotrypsin in Cationic and Nonionic Micellar Media: Ultraviolet and Fluorescence Spectroscopic Approach

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ABSTRACT: α-Chymotrypsin (α-CT) activity was measured in aqueous buffer with the following alkyltriphenylphosphonium bromide surfactants in the series cetyl, tetradecyl, and dodecyl as a tail length. For the sake of comparison with mixed micellar investigation on activity of α-CT, cationic cetyltriphenylphosphonium bromide (CTPB) and nonionic surfactant Triton X-100, Brij-56, Brij-35, Tween 20, and Igepal Co-210 have been used. The *p*-nitrophenyl acetate (PNPA) hydrolysis rate was determined at the surfactant concentration of both cationic and mixed micellar systems by a UV–vis spectrophotometer. The catalytic reaction follows the Michaelis–Menten mechanism, and the catalytic efficiency (k_{cat}/K_M) was evaluated for both homogeneous and mixed-micellar media. The maximum catalytic efficiency was observed at 5 mM concentration of CTPB, but the highest catalytic efficiency, 572 M^{-1} s^{-1}, was measured in the presence of mixed micellar (7.5 mM CTPB + 2.5 mM Tween-20). The fluorescence (FL) spectra showed the differences of α-CT conformations in the presence of cationic surfactants. The FL results suggest that the influence of cationic surfactant on proteolysis arises from the interaction with the α-CT. The binding constant, k_{SV}, of α-CT with cationic aggregates was

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determined in the buffer using the Stern–Volmer equation by the fluorescence spectroscopic approach. © 2015 Wiley Periodicals, Inc. Int J Chem Kinet 48: 79–87, 2016

INTRODUCTION

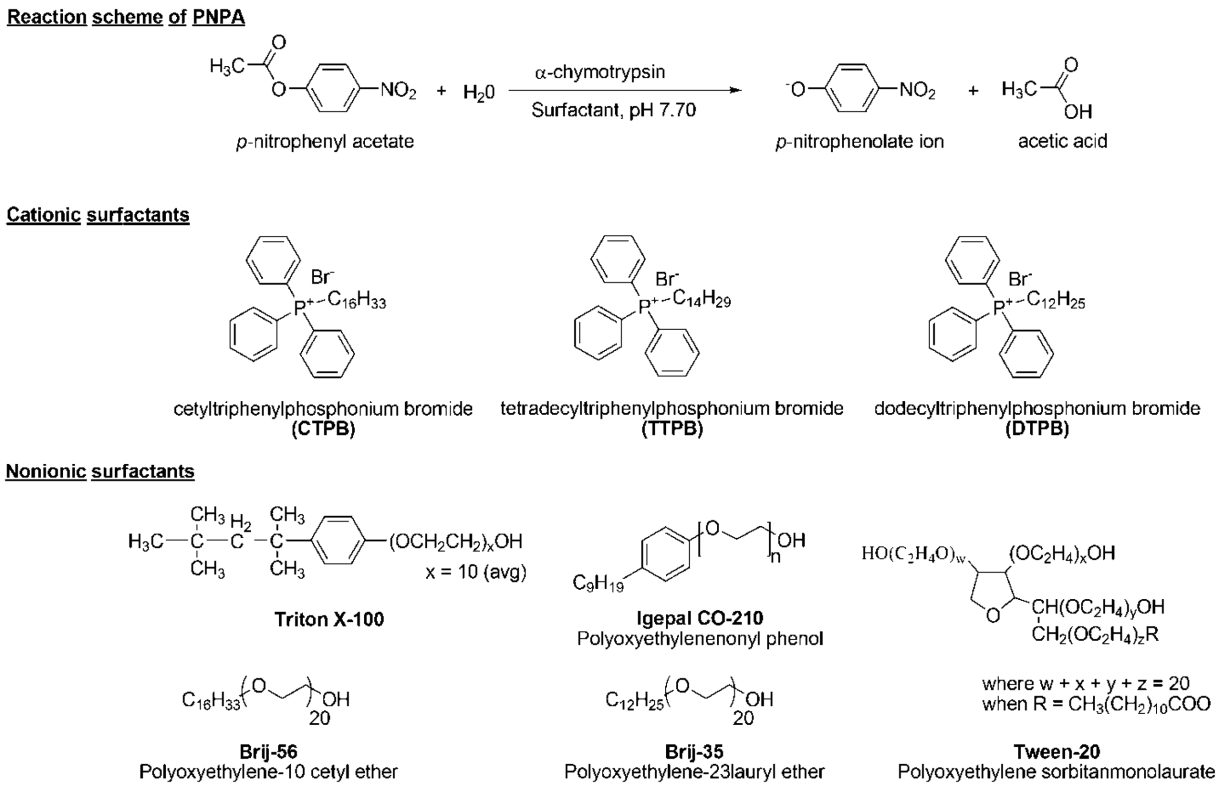
The activity and stability of water-soluble enzymes in aqueous micelles is closely related to the chemical nature of the surfactant. The kinetics and some conformational investigation have already been reported for proteins by fluorescence [1] and UV spectroscopy [2]. The activity of enzyme can be improved by interaction of the α-chymotrypsin (α-CT) with the micelles using surfactants at concentrations above the critical micellar concentration (CMC) [3]. In fact, the interaction between surfactants and proteins in aqueous solutions can be rather specific, the residual enzyme activity being dependent on the nature of both surfactant and enzyme [4]. Alfani et al. [5,6] have carried out numerous studies on the α-CT–catalyzed hydrolysis of *N*-glutaryl-L-phenylalanine *p*-nitroanilide in the presence of cetyltrimethylammonium bromide (CTAB) surfactants with different alkyl groups, which indicate that the α-CT activity increases with increasing size of the alkyl head groups. Nevertheless, the changes in the head group size of surfactant can alter the micellar surface area and the electrostatic interactions of α-CT/surfactant and subsequently affect the degree of ionization and the surroundings of microenvironment. However, an alternative approach has been carried out to minimize above factors to modify the alkyl tail length of surfactant, which can vary the characteristics of the micellar interface [7].

The most important function of nonionic surfactants is to stimulate the catalytic activity of most enzymes. For example, Brij 35 stimulated 1.5-fold cytoplasmic glycerol-3-phosphate dehydrogenase [8], deoxycholate, and many bile salts activated four- to sevenfold alcohol dehydrogenase from rat liver [9], Tween 20 or micellization of substrates resulted in a three- to sixfold increase in the activity of mitochondrial carnitine palmitoyltransferase [10]. Furthermore, it is necessary to study the catalytic activity of α-CT in the presence of cationic and nonionic mixed surfactants system.

The fluorescent probe (e.g., tryptophan, DNA strands) has been used to study the catalytic activity measurement and conformation changes in enzyme. The tryptophan fluorescence (FL) is very sensitive to solvent polarity for the protein–surfactant complex formation. Thus, the emission spectrum of tryptophan residues in proteins is strongly influenced by their environment. This information can be useful in providing an indication of the location of these residues in proteins, as well as affording methods to study association reactions and denaturation [11,12]. The FL arises uniquely from its tryptophan residues when selectively excited at 280 nm. Analysis of the effect of quencher concentration upon protein FL (Stern–Volmer plots) can supply information on quenching constants and estimation of tryptophan accessibility [13]. Celej et al. [14] reported that the catalytic behavior of α-CT affected with the increasing of CTAB concentration showing bell-shaped profile. It is clearly indicating that the micelle-bound enzyme reacts with the free substrate. The α-CT interaction with CTAB changes the tertiary structure of enzyme shows red shift in tryptophan fluorescence spectrum, signifying the termination of internal quenching and a more polar location of tryptophan residues.

In previous works, we reported that the enzymatic activity of α-CT on the hydrolysis of *p*-nitrophenyl acetate (PNPA) strongly depends upon the reaction medium such as oil-in-water microemulsions [15], organic cosolvents [16], cationic surfactant media [17–19], and reverse micelles [20]. Recently, we reported surface and conformational behavior of protein–surfactant interaction by using trypsin and α-CT in cetyltriphenylphosphonium bromide (CTPB) micellar media [21]. We noticed that the tryptophan probe and CTPB molecules adsorb in specific sites on the protein and probably change in micelle-like cluster, which is due to the ionic nature of both protein and surfactant.

The present study was undertaken to investigate the protein and surfactant interaction activity in micellar media and its conformational behavior upon alkyltriphenylphosphonium bromide (cetyl, tetradecyl, dodecyl) surfactants. The proposed reaction scheme for the hydrolysis of PNPA catalyzed by α-CT in the presence of cationic and nonionic surfactants is presented in Scheme 1. Special emphasis has been given in this investigation on the α-CT activity in the cationic surfactants and mixed micellar prepared by a fixed molar ratio of CTPB and nonionic surfactants (Triton X-100, Brij-56, Brij-35, Tween 20, and Igepal Co-210). The kinetic parameters were calculated and discussed. The FL measurements were carried out to study the conformational analysis of α-CT upon the cationic surfactant (CTPB, TTPB, and DTPB). The results of the present study may be of productive use in understanding the interaction of α-CT with cationic (CTPB, TTPB, DTPB) and nonionic surfactants for expanding the mixed micellar application.



Scheme 1 α-CT–catalyzed hydrolysis of PNPA and chemical structure of surfactants.

EXPERIMENTAL

Materials

α-CT (type-II, from bovine pancreas, molecular mass 25 kDa and an isoelectric point pI 8.8) was procured from Sigma (St. Louis, MO) and used without further purification. The cationic surfactant, i.e. alkyltriphenylphosphonium bromide (R = C_{16}, C_{14}, C_{12}), was obtained from Prof. R. M. Palepu, St. Francis Xavier University, Antigonish, Canada, as a gift. The nonionic surfactant, i.e., Triton X-100, Igepal CO-210, Tween-20, Brij-35, Brij-56, were procured from Sigma. The substrate PNPA was procured from Sisco (Mumbai, India). Enzyme and substrate solutions were freshly prepared in the appropriate buffer immediately, before their use in the experiment. Tris-(hydroxymethyl)aminomethane (Tris) (pK_a 8.3) and HCl were obtained from Qualigens (Mumbai, India).

Solution Preparation

The α-CT solution was freshly prepared in acetate buffer pH 4.7 (0.1 M CH_3COOH and 0.1 M CH_3COONa) immediately before their use in the experiments. To improve the solubility of a substrate, the PNPA was prepared in pure acetonitrile (CH_3CN). The presence of acetonitrile does not modify the enzyme–substrate interaction. For a reaction mixture, Tris buffer pH was fixed at 7.70 using 0.1 M hydrochloric acid.

Enzyme Kinetics Measurements

All the spectral measurements were performed using a Varian Cary 50 UV–vis spectrophotometer equipped with a Peltier temperature controller unit at 25°C. The hydrolytic activity of α-CT toward PNPA was monitored by following the increase in the absorbance at 400 nm due to the formation of *p*-nitrophenolate ion (PNP^-) at pH 7.70. All kinetic reaction has been carried out with maintaining 0.065 to 0.52 mM and 13 μM concentrations of PNPA and α-CT, respectively. The obtained p-nitropenolate ion product extinction coefficient was 12,000 M^{-1} cm^{-1}. The initial reaction rate, V_0, was determined from the slope of the PNP^- concentration versus time profiles using enzyme kinetics software (Varian). The rates of all enzyme–catalyzed reactions were corrected for the rate of spontaneous nonenzymatic hydrolysis (buffer/surfactants) determined under identical conditions. The Kinetic

Table I Effect of Chain Length of Cationic Surfactants on α-CT Catalyzed Reaction of PNPA

Surfactant	CMC (mM)	[Surfactant] (mM)	$10^2 k_{cat}(s^{-1})$	$10^3 K_M$ (M)	k_{cat}/K_M ($M^{-1}s^{-1}$)
Buffer		–	0.51 ± 0.02	0.20 ± 0.10	23.1
CTPB	0.16 ± 0.01	1.0	2.03 ± 0.07	0.80 ± 0.01	25.3
		3.0	4.28 ± 2.04	0.76 ± 0.40	56.3
		5.0	5.39 ± 0.26	0.70 ± 0.01	77.0
		10	0.72 ± 0.03	0.10 ± 0.01	72.0
		20	0.59 ± 0.10	0.30 ± 0.01	19.6
TTPB	0.55 ± 0.01	1.0	1.21 ± 0.12	0.25 ± 0.02	48.4
		3.0	1.46 ± 0.17	0.23 ± 0.01	63.4
		5.0	1.52 ± 0.06	0.20 ± 0.01	76.0
		10	1.14 ± 0.06	0.22 ± 0.01	51.8
		20	0.59 ± 0.11	0.24 ± 0.01	24.5
DTPB	1.81 ± 0.02	1.0	1.09 ± 0.06	0.40 ± 0.01	27.2
		3.0	1.20 ± 0.08	0.24 ± 0.02	50.0
		5.0	1.24 ± 0.04	0.20 ± 0.01	62.0
		10	0.95 ± 0.10	0.28 ± 0.04	33.9
		20	0.58 ± 0.07	0.30 ± 0.01	19.3

[α-CT] = 0.02 mM, [Tris/HCl] = 10 mM, temperature = 25°C, pH 7.70, [PNPA] = 0.02–0.20 mM.
*CMC values are taken from [24].

parameters, catalytic constant, k_{cat}, and Michaelis constant, K_M, in micellar solution were obtained by the linear regression analysis of Lineweaver–Burk plot (Eq. (1)).

$$[\alpha - \mathrm{CT}]/V_0 = 1/(k_{cat}) + \left(K_M/k_{cat}\right)[\mathrm{PNPA}]^{-1} \quad (1)$$

FL Measurements

The FL emission spectra of a tryptophan residue in α-CT–surfactant systems were measured using a Varian Carry Eclipse FL spectrophotometer with a slit width of 0.5 cm. The excitation wavelength was 295 nm with bandwidths of 5 nm for excitation and emission. The spectra of 0.54 μM α-CT were obtained at different incubation times of the protein in 20 mM Tris/HCl, pH 7.70 at 25°C, in the absence or presence of increasing surfactant concentrations. Slow changes in the FL spectrum of α-CT were observed up to 20 min incubation with alkyltriphenylphosphonium bromide (C_{16}, C_{14}, C_{12}) micelles. The binding constant of enzyme-surfactant interaction were estimated by the FL measurements using the Stern–Volmer equation (Eq. (2)) [22,23].

$$I_0/I = 1 + K_{sv}[\mathrm{Surfactant}] \quad (2)$$

where I_0 and I are the FL intensities without and with surfactants in the presence of α-CT and K_{sv} is the α-CT surfactant binding constant in protein medium.

RESULTS AND DISCUSSION

Hydrophobicity of Cationic Surfactants on the Enzymatic Activity of α-CT

α-CT activity was measured initially in aqueous buffer (10 mM Tris–HCl, pH 7.70) and in the presence of cationic surfactants at different concentrations above the CMC value (CMC values are taken from [24]). The enzyme kinetic study was undertaken to investigate the effect of cationic surfactants CTPB, TTPB, and DTPB on the α-CT activity in aqueous system, which is enhanced the hydrolysis rate of PNPA (Table I).

As clearly manifested in Table I, the catalytic efficiency (k_{cat}/K_M) of α-CT has been observed to increase with the increasing surfactant from 1 to 5 mM concentration but readily falls at higher concentrations up to 20 mM in case of all cationic surfactants. In comparison with the aqueous buffer, the micelle-bound α-CT showed higher catalytic efficiency for all the three cationic surfactants. The results are most pronounced in CTPB, k_{cat} increases from 2.03×10^{-2} to 5.39×10^{-2} s^{-1} for 1–5 mM and further decreases $0.59 \pm 0.10 \times 10^{-2}$ s^{-1} for 20 mM of the surfactant concentration. On the contrary, K_M values decrease from 1 to 5 mM and further rises up to 20 mM of the surfactant concentration.

Similar trends of α-CT activity are observed for TTPB and DTPB. The catalytic activity for micelle-bound α-CT was raised up to 10.56-, 2.98-, and 2.43-fold for CTPB, TTPB, and DTPB, respectively, than the aqueous buffer. Here, the increase in the catalytic activity of α-CT with increasing hydrophobicity of

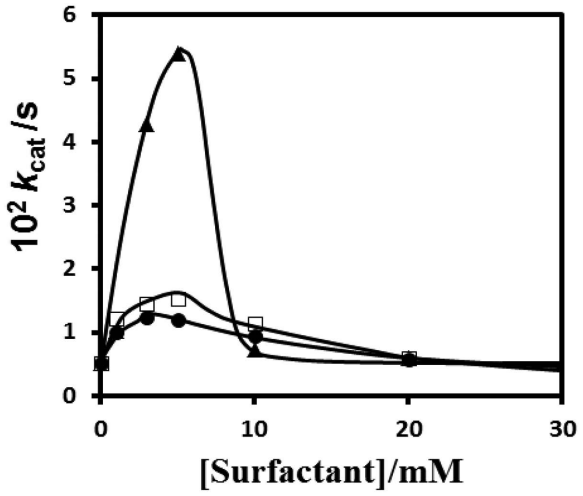


Figure 1 Dependence of the catalytic rate constant on the surfactant concentration. Symbols indicate (▲), CTPB; (□), TTPB; (●), DTPB.

surfactant is due to the enhancing the micellar interface. Further increased interface strongly affects the enzyme activity and providing enhanced microenvironment to aggregate higher concentration of enzyme at the micellar surface [25].

In the presence of CTPB aggregates, the α-CT activity depends markedly on the surfactant concentration: Bell-shaped curves of k_{cat} versus surfactant concentration were obtained. For all surfactant concentrations, the addition of TTPB and DTPB surfactants leads to a decrease in α-CT activity. The full line in Fig. 1 shows reasonable agreement with the experimental data. The α-CT–micelle interaction leads to an increase in both the k_{cat} and the affinity for the substrate PNPA, indicating higher catalytic efficiency for micelle-bound α-CT. Figure 1 also shows the effect of the surfactant concentration on the catalytic activity of α-CT. The α-CT–micelle interaction leads to an increase in both k_{cat} and catalytic efficiency for the substrate PNPA, indicating higher catalytic efficiency for bound α-CT. The bell-shaped profile of α-CT activity with increasing surfactant (CTPB, TTPB, and DTPB) concentration suggests that the micelle-bound α-CT reacts with the free PNPA [14]. These results confirm that the dependence of k_{cat} on the surfactant concentration is similar to that of the catalytic efficiency, being the increase above the CMC higher than the surfactant having the shorter tail length.

Enzymatic Efficacy of α-CT on Cationic and Nonionic Mixed Micellar System

The activity of α-CT was estimated in several mixed micelles prepared from CTPB and nonionic surfactants, Brij-35, Brij 56, Tween-20, Triton X-100, and Igepal CO-210 using 10 mM Tris/HCl buffer (pH 7.70) at 25°C. Mixtures of cationic and nonionic surfactants form mixed micelle aggregates. The properties of mixed surfactant systems are quite different from those of a single individual surfactant system. The results presented in Table II show that, irrespective of the nature of the nonionic surfactants, α-CT entrapped in aqueous buffer of mixed micellar concentration in the ratio (7.5 + 2.5) mM showed 1.02, 2.07, 1.49, and 1.59 times higher catalytic activity for system 2, 6, 10, and 14, respectively, and for mixed micellar system 18 exhibited a less improved α-CT activity (0.90-fold) compared to system 1 containing only CTPB. The interactions between cationic and nonionic surfactants in a mixed micelle result in synergistic effects on the micellization of mixed surfactant systems. Thus, it is quite clear that the nonionic surfactants definitely play an important role in regulating the α-CT activity. The increasing amounts of nonionic surfactants possibly help in reducing the positive surface charge density at the micellar interface. As a consequence, the α-CT activity was boosted up in the mixed micellar systems compared to CTPB [26].

Conformational Analysis of α-CT upon Cationic Surfactants

The crystallographic study of atomic structure of tosyl-[27] and native-α-CT [28] confirmed that two tryptophan residues (Trp-27 and -29) are located in the interior area of the α-CT molecule and the rest of them (Trp-51, -141, -172, -207, -215, and -237) are located at the surface of the α-CT. In the present investigation, the FL study has been done to explore the conformational changes in α-CT in the presence of the cationic surfactant at pH 7.70. The emission spectrum of the native α-CT upon excitation at 295 nm showed a peak at 339 nm for CTPB and 333 nm for both TTPB and DTPB, indicating that the fluorophore of α-CT such as tryptophan at 295 nm was noticeably shielded from the cationic micelles in the native conformation. The least part of the surface area tryptophan residues is accessible to the external solvent [27]. Consequently, it may be presumed that when the surfactant composition is changing the emission wavelength of α-CT has been affected by both conformational changes of the α-CT and changes in surfactant polarity.

Winter and co-workers analyzed using FT-IR spectroscopy and synchrotron small-angle X-ray diffraction that the protein aggregation occurs at relatively low temperatures and at low α-CT concentration [29]. Furthermore, the incorporation of α-CT in aqueous solutions of micelles is less ordered or partially ordered

Table II Summary of α-CT Catalyzed Hydrolysis of PNPA in Cationic and Nonionic Mixed Micellar Media

System	Surfactant	[Surfactant] (mM)	$10^2 k_{cat}(s^{-1})$	$10^3 K_M$ (M)	k_{cat}/K_M ($M^{-1}s^{-1}$)
1	CTPB	10	3.59 ± 0.01	0.20 ± 0.01	180
2	CTPB + TX-100	7.5 + 2.5	3.68 ± 0.80	0.23 ± 0.30	160
3		5.0 + 5.0	1.49 ± 0.04	0.29 ± 0.01	51.3
4		2.5 + 7.5	1.04 ± 0.06	0.34 ± 0.01	30.5
5		0 + 10	0.91 ± 0.05	0.40 ± 0.01	22.7
6	CTPB + Brij-35	7.5 + 2.5	7.43 ± 0.66	0.27 ± 0.10	200
7		5.0 + 5.0	5.53 ± 0.24	0.43 ± 0.01	128
8		2.5 + 7.5	4.57 ± 0.23	0. 60 ± 0.02	76.1
9		0 + 10	1.87 ± 0. 06	0.68 ± 0.02	27.5
10	CTPB + Brij-56	7.5 + 2.5	5.35 ± 0.13	0.20 ± 0.01	268
11		5.0 + 5.0	4.13 ± 0.22	0.32 ± 0.02	129
12		2.5 + 7.5	4.21 ± 0.20	0.44 ± 0.01	95.6
13		0 + 10	2.35 ± 0.50	0.57 ± 0.03	41.2
14	CTPB + Tween-20	7.5 + 2.5	5.72 ± 0.16	0.10 ± 0.01	572
15		5.0 + 5.0	2.64 ± 0.17	0.24 ± 0.05	110
16		2.5 + 7.5	1.49 ± 0.06	0.32 ± 0.02	46.5
17		0 + 10	1.14 ± 0.03	0.46 ± 0.01	24.7
18	CTPB + Igepal CO-210	7.5 + 2.5	3.24 ± 0.09	0.40 ± 0.02	81.0
19		5.0 + 5.0	2.91 ± 0.10	0.43 ± 0.02	67.6
20		2.5 + 7.5	0.99 ± 0.01	0.49 ± 0.01	20.2
21		0 + 10	0.87 ± 0.05	0.63 ± 0.01	13.8

[α-CT] = 0.02 mM, [Tris/HCl] = 10 mM, temperature. = 25°C, pH 7.70, [PNPA] = 0.02–0.20 mM.

structure conformation. The influence of α-CT incorporation on the cationic micellar system resulted in a redshift of the FL emission maximum by a magnitude about the 15 nm from 339 to 354 nm for CTPB, whereas 11 nm from 333 to 344 nm for TTPB and 4 nm from 333 to 337 nm for DTPB at pH 7.70 (Figs. 2a–2c). This structural shifting suggested that the tryptophan for 295 nm was more exposed to the micellar environment in the unfolded aggregation-competent species of the α-CT [30]. Figures 2a–c show that the larger spectral widths of emission spectra also specified the higher heterogeneity of the tryptophan environments in the unfolded structures of α-CT. The influence of α-CT incorporation on a cationic surfactant, a 1.25- and 1.54-fold increase in the FL intensity was observed for the TTPB and DTPB in comparison the CTPB. The FL emission spectral shift is further evidenced for the protein conformation and interaction of surface of α-CT with the micellar interface.

The UV spectroscopic technique can be applied to examine binding and folding/unfolding of α-CT initiated by cationic surfactant, CTPB. Figure 3 shows the absorption spectra of α-CT-catalyzed hydrolysis of PNPA in aqueous buffer at 400 nm due to the formation of *p*-nitrophenolate ion (PNP^-). The absorption spectra slightly shifted (~8 nm) in the presence of 1 mM of CTPB of the same solution. Apart from that the spectra show a sharp increase in absorbance with interaction of α-CT-CTPB as compared to the pure α-CT with PNPA. This is due to the binding of cationic CTPB to high affinity sites of tryptophan residues on the surface of α-CT. The significant change in the absorbance value of α-CT-CTPB mixture is nearly two times higher than the absorbance of pure α-CT, denoting the structural change or denaturation of the α-CT [31].

Fluorescence Binding Studies of α-CT

To active conformation of water-soluble protein, α-CT, a fraction of the hydrophobic side chains is typically buried in the interior of the molecule. However, one of the difficult aspects of the study of protein–surfactant interaction is the determination of the structure of the protein–surfactant complex. For obtaining a molecular-based understanding, it is important to establish how the different regions of the binding isotherm relate to protein conformational changes induced by the surfactant. According to Turro et al. [32], surfactant binding to protein generally displays four characteristics regions, binding is to specific high-energy sites on the protein. Consequently, a decrease in pH shifts the binding isotherm to a higher concentration for cationic surfactants [33].

Incorporation of molecular probes into aqueous micelles and proteins is affected by parameters such as the CMC, degree of water penetration into these surfactant

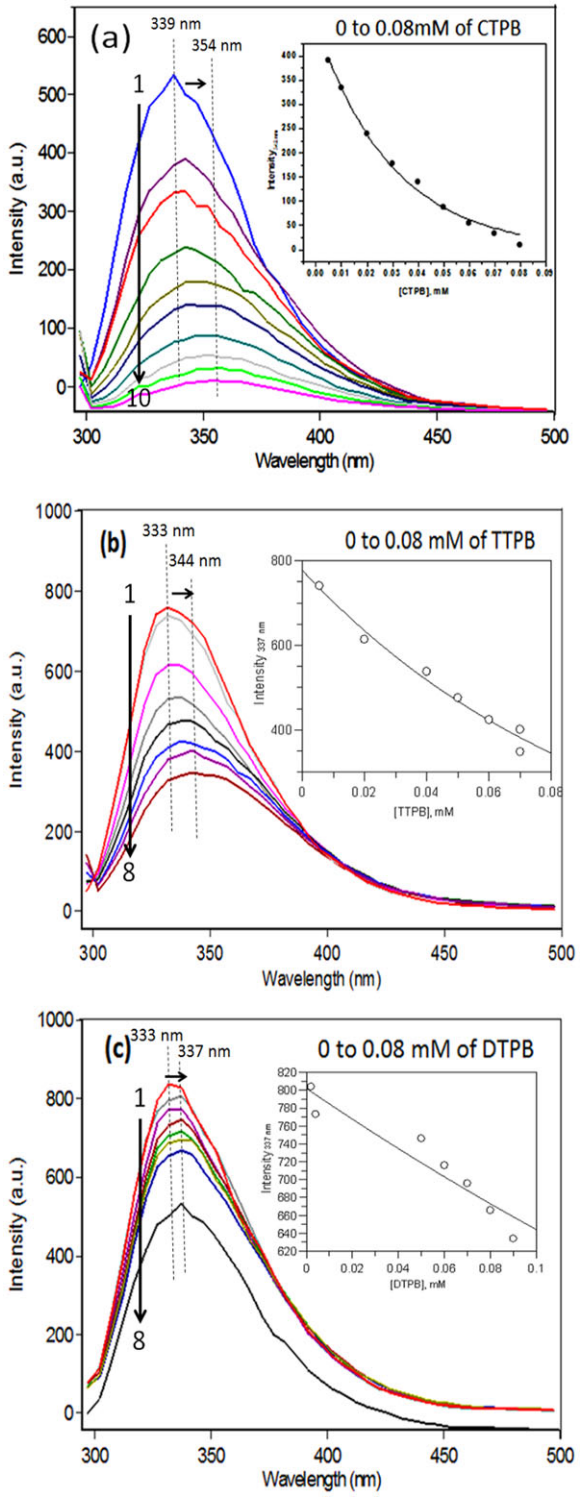


Figure 2 Florescence emission spectra of α-CT and increasing concentration of CTPB (a), TTPB (b), and DTPB (c) at pH 7.70, Temperature 25°C. [α-CT] = 0.54 μM, λ_{exc} = 290 nm, and λ_{emiss} = 337–342 nm. Note: Fig. 2a has been taken from our recently published paper [24].

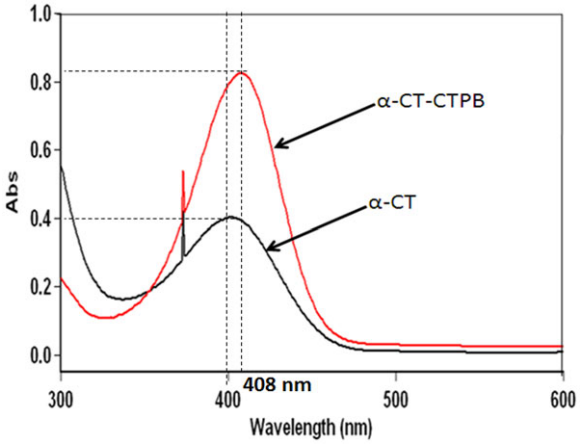


Figure 3 UV–visible absorption spectra of α-CT and α-CT-CTPB micellar interaction in aqueous solution of Tris/HCl buffer at 25°C. [α-CT] = 0.13 μM, [PNPA] = 26 μM, [CTPB] = 1 mM.

Table III Binding Parameter of α-CT-Surfactant Interaction at 25°C

Surfactant	λ_{exc} (nm)	λ_{emiss} (nm)	λ_{max} (nm)	K_{sv} (M^{-1})
CTPB	290	337	342	76718 ± 321
TTPB	290	332	337	16168 ± 189
DTPB	290	332	337	3818 ± 175

aggregations, and local polarity of the microenvironment of the binding sites of proteins [34]. Tryptophan is a novel molecular reporter with potential advantage for following surfactant-induced protein unfolding as well as micelle formation. Thus, FL spectral intensity signatures from a location of the probe in the bulk solvent as well as within the various microenvironments of a surfactant aggregation and protein are discernible. Both the polarity-dependent spectral shift and FL intensity variation enable the FL contributions from multiple populations of probe molecules to be easily resolved.

The FL quenching of α-CT in cationic micellar solutions and water was carried out, and the Stern–Volmer plots I_0/I against the surfactant concentration are found to be linear. A representative Stern–Volmer plot is given in Fig. 4. The binding constant, k_{sv}, of enzyme–surfactant interaction has been determined by the FL intensity data using the Stern–Volmer equation (Eq. (2)), which is reported in Table III. We assume this method can estimate the binding constant for the association of the α-CT with the micelles because the FL intensity of the probe is significantly different from both the environment water and micellar. The binding constant obtained for CTPB with α-CT is higher

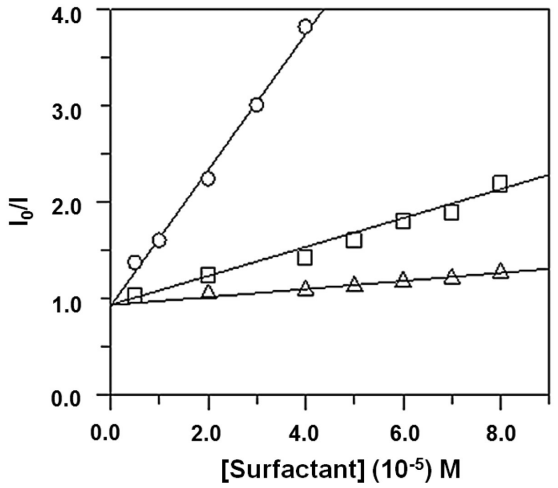


Figure 4 Stern–Volumer plot for α-CT interaction with CTPB (○), TTPB (□), and DTPB (Δ).

compared to those for TTPB and DTPB. This is due to the electrostatic attraction between the α-CT and the charged surface of the cationic micelles, which creates a very high quencher concentration in the vicinity of the probe and causes an efficient quenching [7].

The number of specific binding site increases with hydrocarbon chain length for the interaction between α-CT and a homologous series of *n*-alkyltriphenylphosphonium bromide; the specific binding may occur at lower surfactant concentration with increasing chain length. The order of the binding constant for α-CT with a surfactant has been assumed to follow the following trend: CTPB > TTPB > DTPB. The spectral shifts and enhancement of the FL intensities can be explained in terms of binding of these compounds to fewer polar sites of the micelles (Figs. 1a–1c). In view of a possible intramolecular charge transfer nature of the excited state of α-CT, it is expected that the lowering of the polarity of the medium will destabilize the excited state more than the ground state. As a result, the energy gap between the excited state and the ground state will increase [28]. Thus, the λ_{max} is shifted to the red region. The greater binding constant for neutral species in CTPB than that in TTPB and DTPB indicates that the former micelle provides a more hydrophobic environment to the probe.

CONCLUSION

In summary, we present UV–vis and FL spectroscopic investigation of the interaction of cationic and nonionic surfactant with α-CT. The experimental results show that with increasing hydrophobicity of alkyltriphenylphosphonium bromide (R = C_{16}, C_{14}, C_{12}), the catalytic activity of α-CT was enriched due to the enhancing micellar interface which strongly boosts enzyme activity. The α-CT activity was boosted up in the mixed micellar systems, showing that the nonionic surfactant plays a significant role in regulating the α-CT activity. Information on the nature of α-CT-surfactant binding can be obtained from FL probe studies such as tryptophan residues. This study revealed that emission spectra shifted to higher wavelength in the presence of cationic surfactants (CTPB, TTPB, and DTPB). The FL result is confirmed by considering the hydrophobicity of the surfactants in different concentrations and its association with the enzyme.

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