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α-Chymotrypsin Superactivity in Cetyltrialkylammonium Bromide-Rich Media

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Abstract

α-Chymotrypsin (α-CT) activity was tested with *N*-glutaryl-L-phenylalanine p-nitroanilide in buffered media with added cationic surfactants. The effect of the commercial cetyltrimethylammonium bromide (CTABr) was compared with that of three other surfactants with ethyl (CTEABr), propyl (CTPABr), and butyl (CTBABr) head groups. These were synthesized and purified in this laboratory. Surfactant head groups provided distinct environments that largely modulated the catalytic performance. Larger alkyl head group hydrophobicity led to a marked enhancement of α-CT activity. CTBABr-rich media induced the highest superactivity.

Kinetic measurements were performed in Tris-HCl buffer at a surfactant concentration either below or above CMC, and α-CT superactivity occurred in both media. Positive interactions between the enzyme and surfactants happened independently of the supramolecular organization of the medium. The reaction followed the Michaelis-Menten kinetics. The substrate to micelle aggregates binding constant was used to calculate the substrate concentration available for catalysis. The k_{cat} to K_m ratio was in CTBABr-rich media always higher than in pure buffer and depended on the surfactant concentration. α-CT superactivity depended on the pH value of buffer solution. Enzyme inactivation followed a single-step mechanism in pure buffer and a series mechanism in the presence of a surfactant. The rate of activity decay obeyed a first-order kinetics.

Index Entries: α-Chymotrypsin; superactivity; stability; cationic surfactants; micellar aggregates.

Introduction

Only few investigations have been devoted so far to the specific interactions that occur between surfactants and proteins in aqueous solutions

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to clarify their effects on enzyme stability and activity. Results of these studies could contribute to our understanding of the enzyme behavior in reverse micelles. It is a general opinion that the interior of the reversed micelle acts as a microreactor providing a favorable aqueous microenvironment for the enzyme dissolved in the aqueous core of reverse micelle droplets.

Creagh et al. *(1)* in their study on structure and catalytic characteristics of alcohol dehydrogenase in aqueous solutions of surfactants underlined the importance of electrostatic and hydrophobic interactions between the enzyme and surfactant when designing a reverse micelle system. Correlation of inhibiting and denaturing effects of four different surfactants (anionic, neutral, cationic single-chain, and anionic double-chain) on α-chymotrypsin (α-CT) in surfactant aqueous solutions and in reverse micelles was found by Schoemaecker et al. *(2)*. In contrast, no correlations were found for the behavior of various lipases in the two systems.

A debated point is whether the physicochemical properties of the reverse micelle environment (presumably of the structured one), which induce enzyme superactivity, could be achieved also in the aqueous system (direct micelles). Several reports in the literature have witnessed the superactivity of enzymes in reverse micelles in the case of α-chymotrypsin *(3)*, peroxidase *(4)*, acid phosphatase *(5)*, and laccase *(6)* whose catalytic constant was even accelerated some orders of magnitude with respect to the value in aqueous buffer. Numerous models *(7)* have been proposed to explain the enzyme behavior inside reverse micelles. The superactivity has been interpreted by a higher reactivity of the structured water in the micelle as the water pool presents properties substantially different from those of normal bulk water *(8)*. Alternatively, this was assumed to be a result of the relatively high rigidity of the enzyme molecule caused by the surfactant layer *(9)*.

In a previous paper *(10)* we focused our investigation on aqueous buffer solutions containing self-organizing amphiphilic molecules above the critical micelle concentration (CMC). Their effect on α-chymotrypsin activity was determined. α-CT was selected as a model enzyme since this is a widely studied serine protease with a well-known mechanism of action in aqueous media *(11)* and the superactivity is documented with different substrates in reverse micelles *(3,12–17)*. We reported the superactivity of α-CT during the hydrolysis of *N*-glutaryl-L-phenylalanine p-nitroanilide (GPNA) in the presence of some cetyltrialkylammonium bromide aggregates. The surfactant head group size and buffer type mainly modulated the extent of the enzyme superactivity. The highest one was measured in the presence of cetyltributylammonium bromide (CTBABr). The hydrolysis of GPNA obeyed Michaelis-Menten kinetics. The data after correction for the binding of substrate with micellar aggregates showed that k_{cat} significantly increased while K_m exhibited minor changes. Correlation of the experimentally measured reaction rates was made according to the following kinetic assumptions:

1. all the enzyme is in solution because of the electrostatic repulsion between the cationic surfactant and the positively charged protein at pH below the isoelectric point,
2. a large portion of the substrate was segregated by the micelle aggregates as proved by independent binding determinations.

In order to ascertain whether α-CT superactivity was mainly due to the positive interaction of the monomer surfactant with the enzyme and/or to the structured environment, this investigation was undertaken using the same homologous series of cationic cetyltrialkylammonium bromide surfactants as in ref. *10*. α-CT activity was monitored in the presence of either self-organized assemblies or free molecules of surfactants. The enzyme behavior in commercial cetyltrimethylammonium bromide (CTABr) was compared with three other surfactants, synthesized and purified in the laboratory. The methyl group was replaced with ethyl (CTEABr), propyl (CTPABr), and butyl (CTBABr). The different charge densities and hydrophobicities of the head group could modulate both the interactions with the enzyme and the environment near the protein. The kinetic study also reports the dependence of α-CT superactivity on pH and buffer molarity. The possibility of correlation between the enzyme activity and stability was also investigated.

Materials and Methods

Materials

Sigma (USA) provided the crystalline α-chymotrypsin (EC 3.4.21.1, bovine pancreas, type II, Mw 24.8 kDa, pI 8.8) and the substrate, *N*-glutaryl-L-phenylalanine p-nitroanilide. They were used without further purification. Tris(hydroxymethyl) aminomethane (Tris) was from Aldrich (Germany). The commercial grade surfactant, cetyltrimethylammonium bromide, was from Fluka (Germany) and was purified as reported in ref. *18*. All other chemicals were of analytical grade. The preparation and purification at laboratory scale of the synthesized surfactants cetyltriethylammoniumbromide, cetyltripropylammoniumbromide, cetyltributylammoniumbromide have been fully described in ref. *19*. All surfactants were chemically pure as tested by elemental analysis. The absence of minima in surface tension vs surfactant concentration plots excluded the presence of hydrophobic impurities.

Assay of α-Chymotrypsin Activity

The hydrolytic activity of α-chymotrypsin toward GPNA was measured at 25.0°C using a Shimadzu UV-160A UV-VIS spectrophotometer equipped with a thermostated cell (3 mL vol and 1 cm path length) controlled at ± 0.1°C. The p-nitroaniline (PNA) released during the reaction (10 min) was quantified at 410 nm. The product molar absorptivity was 8750 $M^{-1}cm^{-1}$ either in pure buffer or in the presence of surfactants. Enzyme

activity was typically assayed in 0.1 *M* Tris-HCl buffer, pH 7.75, and with 2.5×10^{-3} *M* GPNA (unless otherwise specified). The reaction was initiated by addition of 60 μL of α-CT stock solution (10 mg/mL). Enzyme and substrate solutions were always freshly prepared in the appropriate buffer immediately before their use. The PNA concentration was then recorded as a function of time. Spontaneous hydrolysis of GPNA did not occur during the incubation period. The specific reaction rate, *r*, defined as moles of PNA formed per unit weight of enzyme (mg) and second, was calculated from the initial linear portion of the concentration vs time curve. The Michaelis-Menten parameters (rate constant, V_{max}, and Michaelis constant, K_m) in only buffer and in buffer plus surfactant were evaluated from the double reciprocal Lineweaver-Burk plot using a linear-least-squares fit program and via a nonlinear regression. For ensuring the accuracy of the findings all sets of experiments, reproduced several times, involved at least duplicates at five different initial substrate concentrations. The turnover number, k_{cat} (s^{-1}), moles of GPNA transformed per second and per mole of enzyme, was then calculated.

Time-Dependent Stability Assays

For inactivation studies of α-CT, solutions of enzyme (1 mg/mL) were prepared and incubated at 25°C separately in glass vessels filled with pure buffer or buffer plus surfactant. Periodically, equal aliquots of the enzyme were withdrawn and mixed with substrate solutions at a constant GPNA concentration. α-CT residual activity was measured as reported in the assay section. No inactivation of the enzyme was detected during the time of kinetic measurements (10 min).

Determination of the Critical Micelle Concentration (CMC)

The CMC of surfactants was determined at 25°C by surface tension measurements. The solutions containing buffer and surfactant at different concentrations were tested in a Krüss du Nouy type tensiometer, according to the standard procedure reported in the literature *(20)*.

Determination of the Binding Constant (K_S)

The following binding equilibrium between GPNA and the surfactant micelles was assumed:

$$[S_w] + [D_n] \overset{K_S}{\Leftrightarrow} [S_M] \quad (1)$$

where S_W stands for the free substrate concentration, S_M for that retained by aggregates, and D_n for that of the micellized surfactant (calculated from the difference between the total surfactant added to the system and CMC). K_S is the equilibrium constant.

The calculation of the binding constant was detailed in ref. *10* and was performed with a least square fit *(21)*:

$$A_\lambda = \frac{(\varepsilon_W + \varepsilon_M K_S[D_n])}{1 + K_S[D_n]} [S_T] \quad (2)$$

where ε_W and ε_M are the molar absorptivities for free and bound GPNA, respectively. Determinations at 328 nm provided the following values in pure buffer, ε_W = 11,500 and in buffer plus surfactant ε_M(CTABr) = 14,300; ε_M(CTEABr) = 14,400; ε_M(CTPABr) = 15,000; ε_M(CTBABr) = 15,300. The binding constant was not affected by buffer molarity.

Results and Discussion

Superactivity as a Function of Alkyl Head Group Size

α-CT activity was measured at the standard temperature, pH, substrate concentration and with 5×10^{-3} *M* surfactant, the concentration largely above CMC value in order to provide self-organizing media. Table 1 quotes specific reaction rates of GPNA hydrolysis both in pure buffer solution, r_b, and in the presence of micelle aggregates, r_{Mb}. The data show that in the experiments carried out at the same overall concentration of the enzyme and substrate α-CT activity can be either depressed by CTABr or significantly promoted by CTPABr and CTBABr. The measured reaction rate, r_{Mb}, in CTABr was 80% of that in pure buffer. When the surfactant alkyl head group size was raised from methyl to butyl residues, the ratio r_{Mb} to r_b increased up to seven times. However, the values in the presence of surfactant could be considered normal. GPNA at the investigated pH was negatively charged because of deprotonation, which promotes its binding with the positively charged surfactant micelles. The binding constant, K_s, was used to calculate the effective substrate concentration, S_w, available for catalysis in the presence of surfactant aggregates *(10)*. A unique value of K_s was reported for CTABr, CTEABr, and CTPABr, since the variations were within the experimental error and no trend was observed with the head group size. The S_w values, together with those of K_m (0.39 m*M*) and k_{cat} ($1.46\ 10^{-2}$/s) determined for GPNA hydrolysis in pure buffer, allowed us to calculate r_b^*, the reaction rate in pure buffer at the effective substrate concentration in each surfactant medium. The enzyme superactivity, ratio of reaction rate with (r_{Mb}) and without surfactant (r_b^*), resulted in all the systems.

Since CTBABr was found to be the most important promoter of α-chymotrypsin activity, other parts of the study were restricted to this surfactant only.

Role of CTBABr on α-CT Kinetics

The r_{Mb}/r_b^* data so far presented are implicitly based on the two assumptions stated in the introduction. However, no direct evidence is yet available, and the hypotheses should be verified. Therefore, experiments were planned to confirm the existence of α-CT superactivity at CTBABr concentration below CMC. This condition ensures that all the substrate is

Table 1
Specific GPNA Hydrolysis Rate as a Function of Surfactant Head Group Size

Surfactant	Experimental reaction rate μmoles/min/mg$_E$	r_{Mb}/r_b	K_S (M^{-1})	S_W (mM)	Calculated reaction rate (r_b^*) (μmoles/min/mg$_E$)	r_{Mb}/r_b^* (dimensionless)
none	0.030	–	–	2.50	–	–
CTABr	0.024	0.80	2000	0.227	0.013	1.85
CTEABr	0.030	1.00	2000	0.227	0.013	2.31
CTPABr	0.098	3.27	2000	0.227	0.013	7.54
CTBABr	0.209	6.97	1500	0.294	0.015	13.93

Experimental conditions: 25°C, Tris-HCl 0.1 M, pH 7.75, 8 μM α-CT, analytical substrate concentration $2.5 \times 10^{-3} M$, surfactant concentration $5 \times 10^{-3} M$.

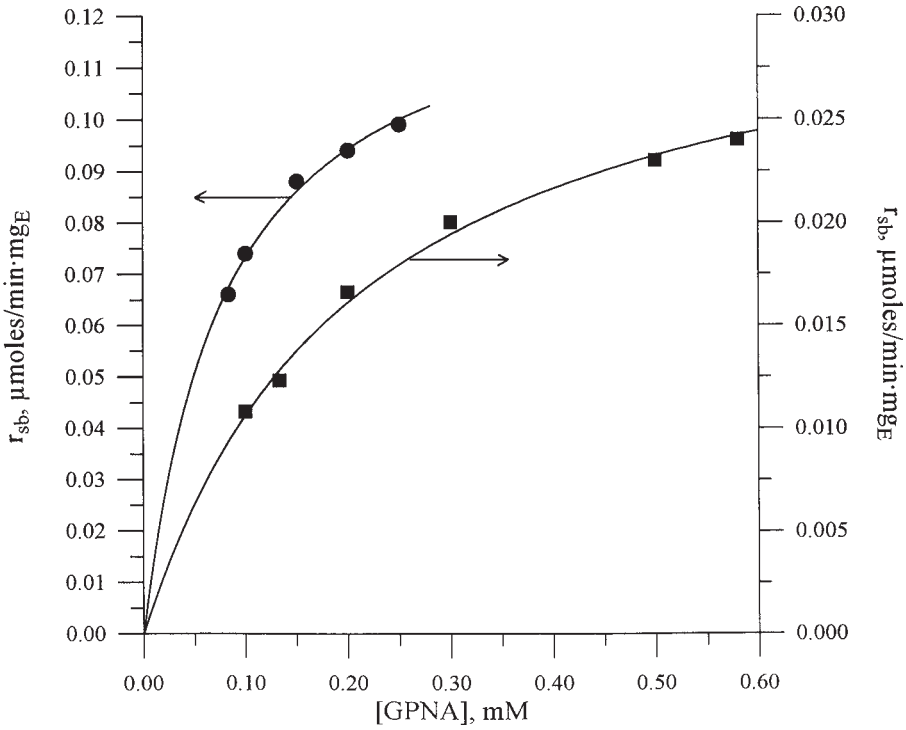

Fig. 1. Rate of GPNA enzymatic hydrolysis vs substrate concentration. T = 25°C, pH = 7.75, α-CT = 8 μ*M*: ● $5 \cdot 10^{-5}$ *M* CTBABr in 0.015 *M* Tris-HCl, ■ $5 \cdot 10^{-5}$ *M* CTBABr in 0.1 *M* Tris-HCl.

available for catalysis and the activity of free enzyme could be affected only by the surfactant monomer.

For the sake of comparison, the effect of CTBABr on the enzyme activity was investigated at two principal concentrations of the surfactant: 5×10^{-3} *M* (presence of micelle aggregates) and 5×10^{-5} *M* (likely presence of only surfactant monomers). In ref. *10* it was proved that buffer concentration was a key factor for the level of α-CT superactivity in the presence of micelle aggregates. Enzyme activity continuously decreased with increasing buffer concentration. Therefore, the kinetic determinations below surfactant CMC were performed in Tris-HCl buffer at both 0.1 *M* and 0.015 *M*. Lower buffer concentrations can be difficult to use because of the limits in substrate solubility. Figure 1 reports the dependence of GPNA hydrolysis rate, r_{sb}, on the substrate concentration in the presence of monomeric CTBABr. Activity of α-chymotrypsin in 0.015 *M* Tris-HCl (left-hand axis) was much higher than that in 0.1 *M* buffer (right-hand axis). The largest observed percentage of rate increase at the lower buffer molarity was 560%. In both systems data were well described by the Michaelis-Menten rate equation. The estimated kinetic parameters indicated that both K_m and k_{cat} depended on buffer concentration.

The results of experiments performed above CMC confirmed that GPNA hydrolysis obeyed Michaelis-Menten kinetics. This remained true

Table 2
Effect of CTBABr on α-CT Kinetics

Surfactant	Reaction medium	[Buffer] *M*	[CTBABr] *M*	K_m (m*M*)	k_{cat} (s)	k_{cat}/K_m (*M*s)
	Tris-HCl buffer	0.1		0.39	1.46×10^{-2}	37.43
	Tris-HCl buffer	0.015		0.33	1.08×10^{-2}	32.73
CTBABr	Tris-HCl buffer	0.1	5×10^{-3}	3.69	19.5×10^{-2}	52.85
CTBABr	Tris-HCl buffer[a]	0.1	5×10^{-3}	0.43	19.5×10^{-2}	453.49
CTBABr	Tris-HCl buffer	0.1	5×10^{-5}	0.21	1.38×10^{-2}	65.71
CTBABr	Tris-HCl buffer	0.015	5×10^{-5}	0.08	5.5×10^{-2}	687.50

Experimental conditions: 25°C, Tris-HCl buffer, pH 7.75, 8 μ*M* α-CT.
[a]Data after correction for free substrate concentration.

for the micellized system (5×10^{-3} *M* CTBABr and 0.1 *M* buffer) also after data correction for the effective substrate concentration (see Materials and Methods for details). The estimated kinetic parameters for the various systems are quoted in Table 2.

The difference in α-CT activity cannot be attributed to variations in the chemical reactivity of the deprotonated GPNA in the presence of the positively charged head group of surfactants. Micellar catalysis *(22–24)* was not effective since no product formation was observed with only surfactant at the concentration above CMC during the time interval usually adopted for the enzyme assay. GPNA was also incubated at 25°C in buffer solution without enzyme but with the various surfactants below CMC. The p-nitroanilide formed was recorded versus time for 120 h and results proved that GPNA hydrolysis was not at all relevant (time for 50% conversion was estimated to be 800 d or longer). Hence substrate reactivity was not affected by surfactant and by the difference of the head group size.

In pure aqueous medium the low concentration of buffer species does not largely modify the charge distribution over the protein surface exhibiting the kinetic parameters almost unchanged. The K_m value of micellized medium, directly determined using the assumption that all the substrate was available for catalysis, was much higher than K_m in the absence of surfactant. Interestingly, the one, $K_{m(Sw)}$, evaluated taking into account substrate partitioning to surfactant aggregates has a value very close to that in pure buffer media. The k_{cat} was not affected by the data correction and remained 13.3 times higher than in buffer. Therefore, the resulting catalytic efficiency ($k_{cat}/K_{m(Sw)}$) was very high.

The presence of monomeric surfactant caused a reduction of K_m value in comparison with those in pure buffer and in buffer plus micelles. This indicates that the enzyme-substrate complex is more easily formed. In 0.1 *M* Tris-HCl buffer k_{cat} remained as in pure buffer but increased 5.1 times in 0.015 *M* buffer. The results of experiments with 0.1 *M* buffer concentration and 5×10^{-5} *M* CTBABr concentration, below CMC, proved that the α-CT catalytic efficiency (65.71) was only two times higher than in pure buffer (37.43) mainly because of the lower K_m value in comparison with the pure aqueous medium. Inspection in Table 2 of the Michaelis parameters calculated from the experiments carried out at the low buffer concentration, 0.015 *M* Tris-HCl, clearly shows that large efficiency of α-CT (687.50), even greater than the one (453.49) observed in the presence of micelle aggregates, can be reached. The improved efficiency of the enzyme in comparison with that in other systems was mainly due to the high affinity toward the substrate (K_m was much lowered) together with a good value of catalytic constant. The body of these findings confirmed that the enzyme superactivity did not depend only on protein-surfactant interactions but involved the buffer as well.

Improvement of enzyme efficiency does not necessary involve micelle formation. At concentrations below CMC hydrophobic interactions between surfactant and enzyme are likely predominant and promote α-CT

activity. This behavior can be explained as consequence of both a more favorable enzyme conformation (higher k_{cat}) and an improved affinity toward the substrate (lower K_m). Buffers with a relatively higher concentration can electrostatically disturb the positive surfactant-enzyme interactions, determining the reduction of enzyme efficiency, as a result of both a lower catalytic constant and affinity toward the substrate (higher K_m). Modification of both enzyme conformation and microenvironment could possibly have been the cause.

The much higher α-CT efficiency measured in micelle systems (453.49) in comparison with that (65.71) in surfactant monomer at the same pH and ionic strength seems to rule out the possibility that the efficiency improvement can be the result only of interactions between the enzyme and the surfactant monomer. On the contrary, the conformation of the enzyme near the structured micelle environments is largely improved (higher k_{cat}) even though the substrate affinity is depressed (higher K_m) is a sound hypothesis.

In order to further verify the importance of surfactant addition to the reaction medium, hydrolysis rate was measured at 25°C in the presence of 0.015 *M* Tris-HCl, pH 7.75, and with increasing amounts of CTBABr below its CMC, 6.3×10^{-5} *M*. The substrate concentration was 0.294 m*M* as S_w during hydrolysis in micelle aggregates. α-CT activity was linearly dependent on CTBABr concentration until the onset of micelle aggregates, as clearly indicated in Fig. 2. The hydrolysis rate in pure buffer under these conditions was 0.012 μmoles/min and per mg of enzyme. The ratio of surfactant to enzyme molecules in the investigated range varied from roughly 1:1 to 5:1. Therefore, very few molecules of surfactant per molecule of α-CT can induce a very important superactivity. The linear extrapolation of the reaction rate vs surfactant concentration function allowed predicting the value at the CMC. It would be 0.12 μmoles/min and per mg of enzyme, a value that is 10 times higher than in pure buffer.

Effect of pH on α-CT Activity

Specific rate of GPNA enzymatic hydrolysis was monitored as a function of buffer pH in order to further investigate the relative importance of hydrophobic and electrostatic interactions. The pH of 0.1 *M* Tris-HCl buffer was varied in the range from 7.5 to 9.0. Figure 3 (right-hand axis) displays pH profiles (open symbols) as percentage of maximum activity in pure buffer and in buffer plus CTBABr 5 m*M*. The shape of curves was different but the optimum pH remained unchanged. In Fig. 3 (left-hand axis) the ratio of the specific reaction rate (closed symbols) in the presence of micellized surfactant to that in pure buffer, r_{Mb}/r_b, was also plotted. The optimum pH was slightly shifted toward a higher value (8.5). Reaction rates in the presence of CTBABr were greater than in pure buffer over the investigated range of pH. Therefore, α-CT superactivity cannot be ascribed only to the pH profile shifts as reported for enzymes confined in water pools of reverse micelles *(3,12,25,26)*.

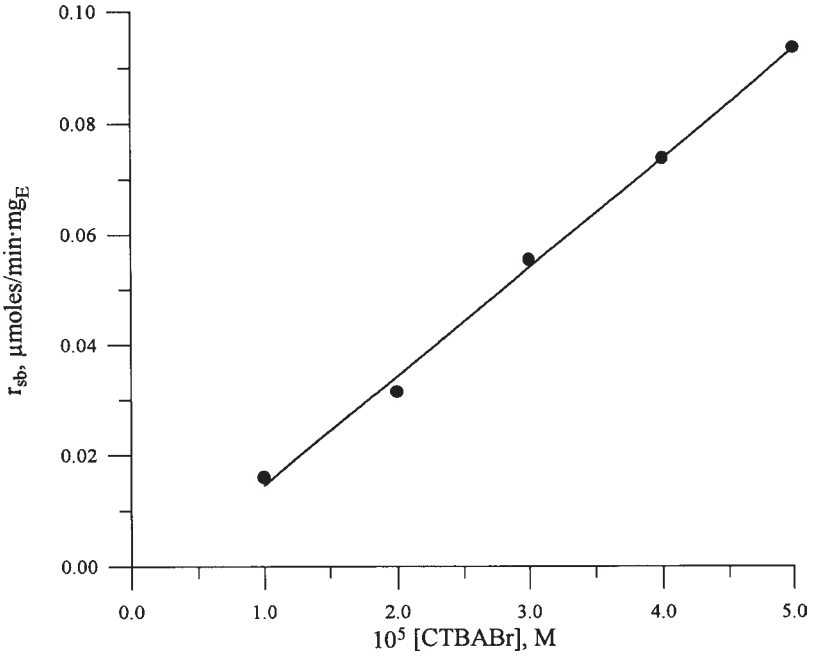

Fig. 2. Rate of GPNA enzymatic hydrolysis versus surfactant concentration. *T* = 25°C, 0.015 *M* Tris-HCl, pH = 7.75, α-CT = 8 μ*M*, GPNA = 0.294 m*M*.

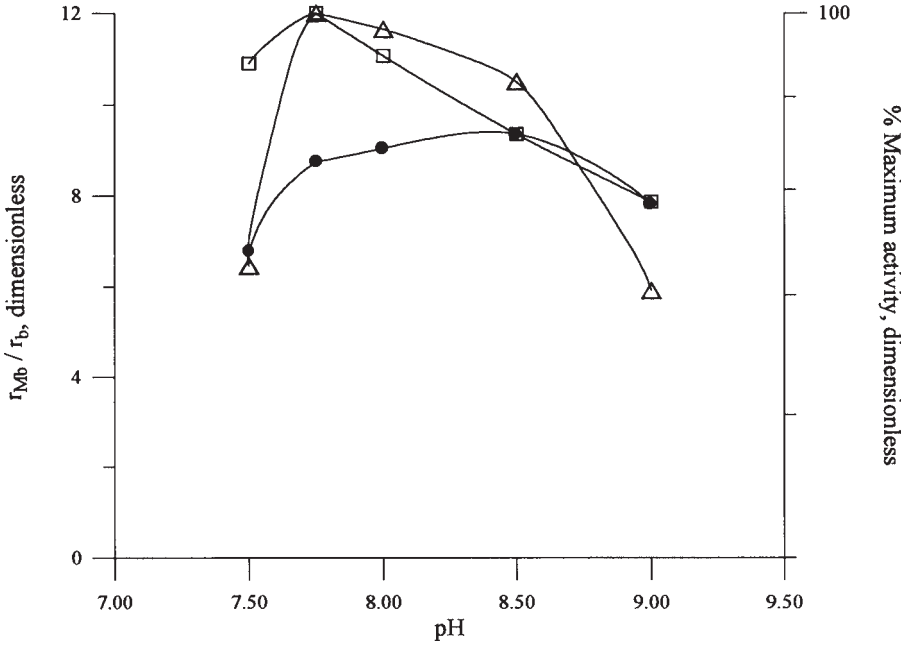

Fig. 3. Effect of buffer pH on α-CT activity in CTBABr above the CMC. *T* = 25°C, 0.1 *M* Tris-HCl, α-CT = 8 μ*M*: ● - r_{Mb} / r_b, open symbols - % maximal activity (□ - pure buffer, △ - buffer plus $5 \cdot 10^{-3}$ M CTBABr).

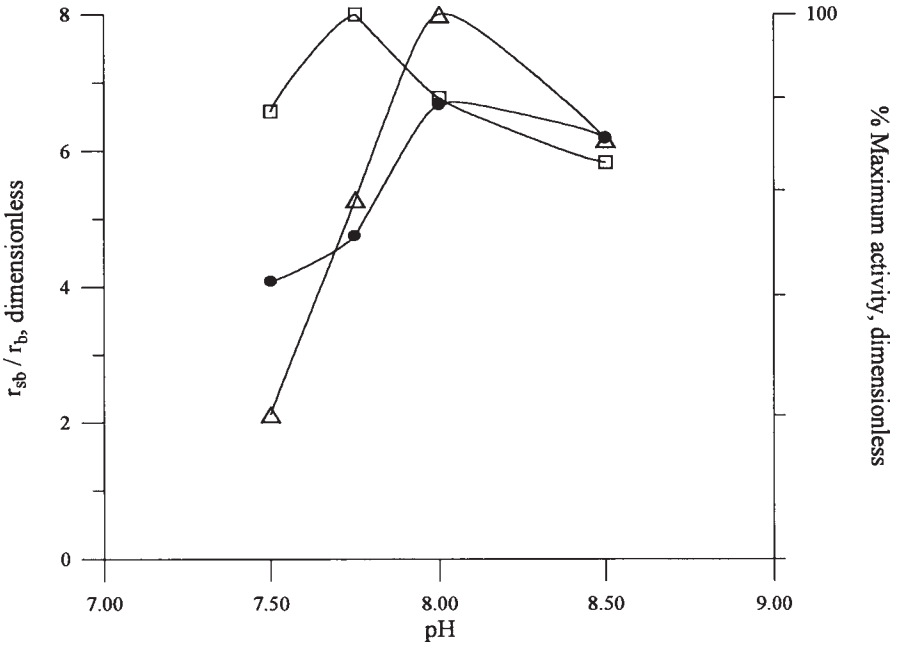

Fig. 4. Effect of buffer pH on α-CT activity in CTBABr below the CMC. T = 25°C, 0.015 M Tris-HCl, α-CT = 8 μM: ● - r_{sb}/r_b, open symbols - % maximal activity (□ - pure buffer, △ - buffer plus $5 \cdot 10^{-5}$ M CTBABr).

Figure 4 depicts the effect of buffer pH on α-CT activity at low CTBABr concentration (5×10^{-5} M) and in 0.015 M Tris-HCl buffer. The buffer pH ranged from 7.5 to 8.5. The data of maximum activity percentage (open symbols) indicate that under these conditions a shift of optimum pH occurred. The presence of monomeric surfactant modified the pH profile, and the optimum pH for α-CT superactivity was 8.0.

The ratio of specific reaction rate, r_{sb}/r_b (closed symbols), still remained much higher than one over the whole pH range, and the largest value was attained at the optimum pH of the enzyme in surfactant-rich medium. The dependence of α-CT superactivity on pH at low ionic strength and with surfactant below CMC was more evident than in systems with high ionic strength and with micellized surfactant. Therefore, we speculated that electrostatic interactions, induced by pH variations, disturbed the enzyme conformation in the structured environment to a lesser extent than in the remaining medium.

Michaelis parameters were evaluated at the optimal pH (8.0) in 0.015 M Tris-HCl buffer. K_m and k_{cat} were 0.11 mM and 9.5×10^{-2}/s, respectively. This clearly indicated the effective beneficial effect of optimal pH on catalytic efficiency (k_{cat}/K_m, 864.0/Ms).

Enzyme Stability

The inactivation kinetics of α-CT was monitored at 25°C in the presence of either pure buffer (0.015 M and 0.1 M Tris buffer, pH 7.75) or buffer

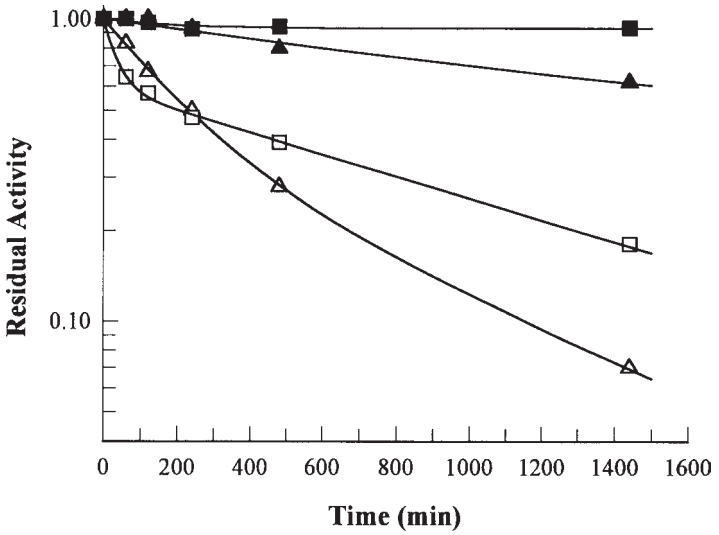

Fig. 5. α-CT residual activity vs storage time. T = 25°C, pH = 7.75, α-CT = 8 μM: ■ - 0.1 M Tris-HCl, ▲- 0.015 M Tris-HCl, □- 0.1 M Tris-HCl plus $5 \cdot 10^{-3}$ M CTBABr, △ - 0.015 M Tris-HCl plus $5 \cdot 10^{-5}$ M CTBABr.

plus surfactant. CTBABr concentrations were 5×10^{-5} M in 0.015 M Tris-HCl and 5×10^{-3} M in 0.1 M Tris-HCl. Residual activity, ratio of instantaneous to initial specific reaction rate, is illustrated in Fig. 5. The semi-log scale was used to show the deviation from the first-order inactivation kinetics. The rate of enzyme activity decay depended on buffer molarity. In 0.015 M buffer it was higher than in 0.1 M buffer. This behavior was also confirmed when surfactant was present in the medium. Furthermore, monomeric surfactant caused the highest rate of α-CT inactivation. Nevertheless, in spite of a faster enzyme inactivation GPNA hydrolysis continued at a higher rate because of the large superactivity induced by the surfactant. Correlation of residual activity vs storage time was found using the series inactivation mechanism model, which assumes the existence of native enzyme, an active intermediate, and a totally denaturated form. a_N and a_i are the specific activities, k_{dN} and k_{dI} are the first-order rate constants of enzyme inactivation for native enzyme and the intermediate, respectively. The calculated values are reported in Table 3. They indicated that in pure buffer the single-step mechanism occurred at a low rate and the rate constant was higher in 0.015 M buffer. On the contrary, in the presence of both micellized and monomeric surfactant, the two-step mechanism holds and the second deactivation step occurred with a significant rate. The relative activity in 0.015 M Tris-HCl buffer and monomeric surfactant was smaller than in the presence of micelles. These different behaviors could be ascribed to some modifications in the protein conformations that make the enzyme more active and at the same time more sensitive to inactivation.

Table 3
Parameters of α-CT Deactivation Mechanism

[Buffer] *M*	[CTBABr] *M*	k_{dN} (h)	a_I/a_N (dimensionless)	k_{dI} (h)
0.1	–	2.58×10^{-3}	–	–
0.1	5×10^{-3}	2.50×10^{-2}	0.57	8.34×10^{-4}
0.015	–	1.99×10^{-2}	–	–
0.015	5×10^{-5}	4.78×10^{-3}	0.30	1.23×10^{-3}

Conclusions

Activity of α-CT in the hydrolysis of GPNA was promoted in the presence of cationic cetyltrialkylammonium bromide surfactants. The extent of superactivity increased with the size of the head group. Both monomeric and micellized CTBABr induced large improvements of enzyme efficiency. However, this occurs in the monomeric surfactant medium when buffer molarity is drastically reduced. The turnover number and K_m (both apparent and the value after data correction) of α-CT near structured micelle environments were higher than those of enzyme interacting with the surfactant monomer. Reaction rates measured using different pH and ionic strength of Tris-HCl buffer proved that enzyme superactivity depended on protein-surfactant-buffer interactions and involved both hydrophobic and electrostatic types. Furthermore, superactivity cannot be ascribed to the shift in pH-optimum only. Comparison of activity and stability measurements suggested that changes in the protein conformation are caused by the presence of surfactants, and α-CT becomes both more active and sensitive to inactivation.

Acknowledgments

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