A Quantitative Analysis of the Effect of Hexadecyltrimethylammonium Bromide Micelles on the Rate of Alkaline Hydrolysis of Benzylpenicillin

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The rate of alkaline hydrolysis of benzylpenicillin (BP) is enhanced by micelles of hexadecyltrimethylammonium bromide. Addition of sodium bromide or increasing the concentration of BP inhibits the micellar rate enhancement. The effects of micelles, salt, and variation of BP concentration have been analysed quantitatively by using a pseudophase model with explicit consideration of ion exchange.

The pseudophase model, with explicit consideration of ion exchange, has been successfully used to analyse quantitatively a series of chemical reactions and equilibria in micellar systems.[1] Renewed interest in the study of models for the hydrolytic degradation of penicillin,[2] and a recent study of micelle-modified hydrolysis of benzylpenicillin[3] (BP), prompted us to re-analyse the effect of hexadecyltrimethylammonium bromide (CTAB) micelles on the alkaline hydrolysis of BP.

The mechanism of hydrolysis of BP involves the (rate-limiting) attack of hydroxide ion to generate a tetrahedral intermediate that decomposes to yield penicillinic acid[4] [reaction (i)]. The analysis of the effect of micelles on this reaction requires a consideration of the mode of distribution of BP and ^-OH between the aqueous and micellar pseudophases. The specific form in which the substrate distribution is analysed conceptually (and mathematically) depends on the relative charges on the substrate and the micelle. In the limit of fast exchange, neutral substrates are distributed between the aqueous phase and the micellar pseudophase as expected for simple solute distribution between the two pseudophases,[5] while substrates bearing the same charge as the micelle can be considered (and analysed) as pseudomonomers.[6] In reaction (i) both BP and the attacking hydroxide should be considered as counterions of the (positively charged) CTAB micelle.[1] Thus, the kinetic effect of CTAB on reaction (i) will reflect the interplay of all factors (salt, detergent concentration, [^-OH], [BP]) which alter the counterion distribution between water and the micellar pseudophase.[7] In this report, we show that the rate enhancement produced by CTAB on reaction (i) can be analysed quantitatively within the framework of the pseudophase ion-exchange model.

Results and Discussion

The u.v. spectra of aqueous solutions of BP were modified upon addition of CTAB (Figure 1A, inset), the magnitude of the spectral changes being sensitive to both CTAB and NaBr (Figure 1). The spectral shifts were relatively small and probably were not noticed previously[3] because of the particular experimental conditions (BP, salt, and detergent concentration) used in earlier work.

Solubilization or binding of several solutes in micelles causes changes in the spectral properties of the chromophores[8] that have been related to the nature of the solubilization sites in the micelles.[9] These spectral shifts have been used both to estimate selected properties of the micellar environment[10] and to evaluate distribution coefficients and selectivity constants for ion exchange.[11]

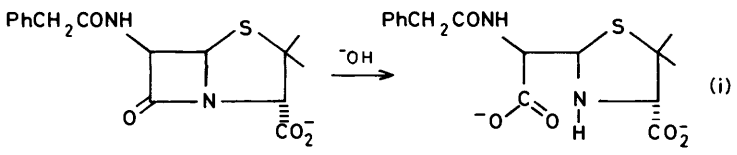
The absorbance (A) of a solution of a chromophore such as BP, which bears a charge opposite to that of the CTAB micelle and exhibits an absorptivity difference between the aqueous (ε_f) and micellar (ε_b) pseudophases, can be expressed[12] as equation (1). In the micellar solution the concentrations of free BP (BP_f)

$$A = \frac{\varepsilon_b + \varepsilon_f K_{BP/Br} \dfrac{[Br_f]}{[Br_b]}}{1 + K_{BP/Br} \dfrac{[Br_f]}{[Br_b]}} \qquad (1)$$

and bound BP (BP_b) are related to those of free bromide ion (Br_f) and bound bromide ion (Br_b) through the selectivity coefficient for ion exchange[1,7] ($K_{BP/Br}$).

The addition of CTAB caused a decrease in the absorbance of solutions of BP (Figure 1A) whereas, at constant [CTAB], the addition of NaBr produced an increase in the absorbance which, as a limit, reached the absorbance of BP in the absence of CTAB (Figure 1B).

The data in Figure 1 were fitted to equation (1) by published procedures,[1] with a multiple regression program on a TRS-80 microcomputer. The value for the degree of counterion dissociation (α) was taken as 0.20,[13] and the critical micelle concentration (CMC) of CTAB in water was taken as 9.20×10^{-4} mol l^{-1} and was corrected, in a separate subroutine, for the addition of salt.[14] The value of ε_f at 256.8 nm was found to be 257 l mol^{-1} cm^{-1} (lit.,[15] ε_{257} 240 l mol^{-1} cm^{-1}), and the best fit values for $K_{BP/Br}$ and ε_b, which were used as variable adjustable parameters, were 6.0 ± 0.5 and 218 ± 10 l mol^{-1} cm^{-1}, respectively. The spectral data demonstrated that: (a) benzylpenicillin binds to the CTAB micelle, (b) the binding produces a shift in the spectra of the chromophore, (c) the spectral shift reaches a constant value upon increasing detergent concentration, (d) BP can be displaced from the CTAB micelle by added bromide, and



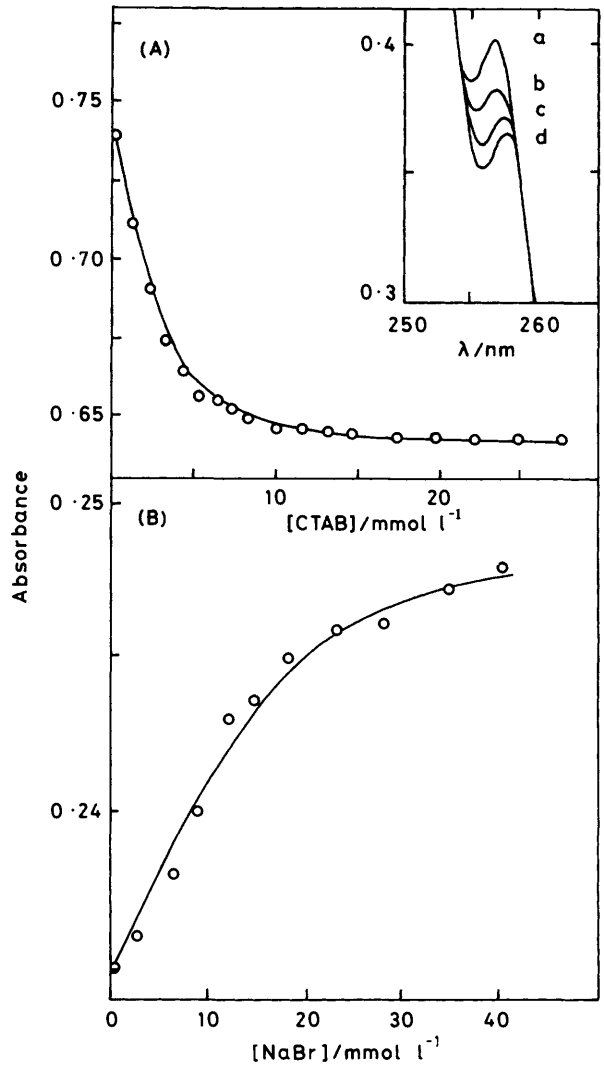


Figure 1. Effect of hexadecyltrimethylammonium bromide on the spectra of benzylpenicillin: (A) absorbance (258.6 nm) of a solution of BP (2.88×10^{-3} mol l^{-1}) as a function of CTAB (solid line calculated; see text); the inset shows the effect of CTAB on the spectra of BP (1.56×10^{-3} mol l^{-1}); CTAB concentrations were: a = 0, b = 9.6×10^{-4}, c = 1.9×10^{-3}, and d 2.4×10^{-3} mol l^{-1}; (B) effect of NaBr on the absorbance of a solution containing 8.0×10^{-3} mol l^{-1} CTAB and 1.0×10^{-3} mol l^{-1} BP (solid line calculated; see text)

(e) the binding of BP and displacement by bromide can be treated as ion-exchange phenomena.

The addition of CTAB, as shown previously,[3] produced an increase in the rate of alkaline hydrolysis of BP. The bell-shaped curves relating the observed pseudo-first-order rate constant (k_ψ) with detergent concentration were extremely sensitive to the addition of NaBr (Figure 2). Curves exhibiting an increase in the rate constant with detergent concentration followed by a decrease at higher detergent concentration are a common feature of micelle-modified bimolecular reactions.[16] The maximum rate acceleration ($k_{max.}/k_{\psi 0}$) [where $k_{max.}$ is the maximum value of k_ψ for reaction (1) in the presence of CTAB and $k_{\psi 0}$ that obtained in the absence of added detergent] in the absence of salt was 53 (0.4 mmol l^{-1} total BP [BP_T], 11.3 mmol l^{-1} OH_T), reduced to *ca.* 10 by the addition of NaBr (21 mmol l^{-1}).

Table. Effect of the concentration of benzylpenicillin (BP) on the rate of hydrolysis of benzylpenicillin in the presence of micelles[a]

10^3[BP]/ mol l^{-1}	$10^2 k_\psi/s^{-1}$	$10^2 k_p/s^{-1}$ [b]	k_ψ/k_p
0.02	5.91 ± 0.4	5.93	1
0.04	5.77 ± 0.4	5.77	1
0.10	4.76 ± 0.5	5.36	0.89
0.20	4.25 ± 0.4	4.71	0.90
0.42	2.65 ± 0.3	3.64	0.73
0.82	1.02 ± 0.08	2.26	0.45
1.23	0.86 ± 0.08	1.47	0.58

[a] [NaOH] = 0.010 mol l^{-1}; [CTAB] = 2.5×10^{-3} mol l^{-1}. [b] Calculated from equation (3) (see text).

Added salts decrease the rate of several micelle-modified alkaline hydrolyses of neutral substrates,[17] and this inhibitory effect has been rationalised in terms of ion-exchange processes whereby hydroxide in the Stern layer is replaced by other counterions.[18] The increase in [BP_T], at constant CTAB and salt, also caused a marked decrease in the value of k_ψ (Table; see also ref. 3). When the substrate is a counterion such as BP an inhibitory effect of increasing substrate concentration can be predicted on the basis of the overall distribution of ions in the system. Since BP binds 75 times better than hydroxide, an increase in [BP_T] should alter the overall ion distribution of the micellar system, diminishing [OH_b] and, as a consequence, decreasing the rate of reaction in the micellar phase. All these kinetic effects can be analysed quantitatively by using the pseudophase-ion exchange formalism.[1] The rate of reaction between ^-OH (excess) and BP in the presence of positively charged micelles can be described by equation (2), where k_ψ, k_2°,

$$v = k_\psi [BP_T] = k_2^\circ [OH_f]_l [BP_f]_l + k_{2m} [OH_b]_l [BP_b]_l \quad (2)$$

and k_{2m} are the observed pseudo-first-order rate constant and the aqueous and micellar second-order rate constants, respectively and 'l' refers to local concentration. By using published procedures,[7] equation (2) can be expressed as (3), where $\bar{V}$ is the partial molar volume of micellised detergent, $k_{OH/Br}$ the selectivity coefficient for $^-OH/Br$ exchange in CTAB, and C_D the concentration of micellised detergent.

The fit of the data in Figure 2 involves the use of equation (3) and the following parameters: $K_{BP/Br} = 6$ (see above), $\alpha = 0.2$,[13] $K_{OH/Br} = 0.08$;[19] $\bar{V} = 0.37$ l mol^{-1},[16] $k_2^\circ = 0.10 \pm 0.02$ (obtained here, coincident with the literature[3]). The best-fit value for the *only* variable parameters k_{2m} was 0.105, identical with k_2° within our experimental error. Similar values of k_2° and k_{2m} are frequent[9] for several reactions.

The inhibitory effect observed upon increasing the concentration of BP on the micelle-modified alkaline hydrolysis (Table) was calculated from equation (3) and all the parameter values used to fit the data in Figure 2 (see before). The agreement between the predicted (k_p) and experimental (k_ψ) values was excellent for the lowest values of [BP_T] and within experimental error up to 2×10^{-4} mol l^{-1} [BP_T] (Table). Above that concentration the predicted values were higher than those

$$k_\psi = [OH_T] \frac{k_2^\circ \left(\frac{[Br_f]}{[Br_b]}\right)^2 + (k_{2m}/\bar{V}) K_{OH/Br} K_{BP/Br} (1/C_D)}{\left(1 + \frac{[Br_f]}{[Br_b]} \frac{1}{K_{OH/Br}}\right)\left(1 + \frac{[Br_f]}{[B}\, r_b] \frac{1}{K_{BP/Br}}\right) K_{BP/Br} K_{OH/Br}} \quad (3)$$

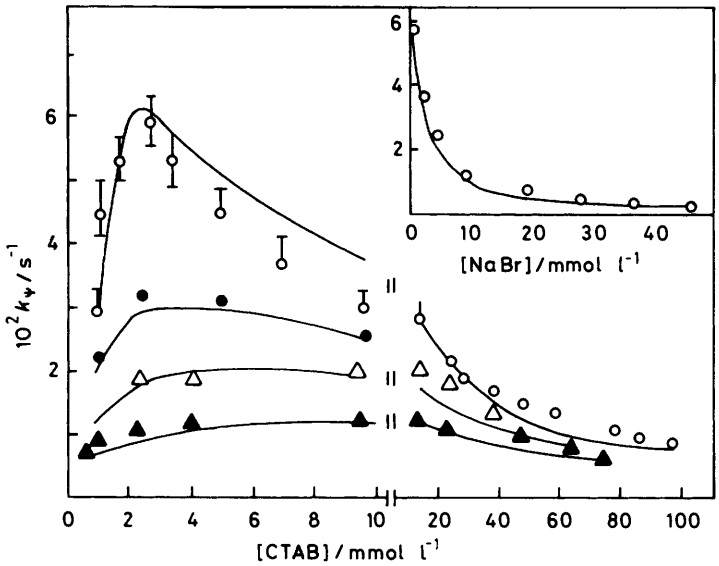


Figure 2. Effect of hexadecyltrimethylammonium bromide on the rate of alkaline hydrolysis of benzylpenicillin; [BP] 4.2×10^{-4}, [NaOH] 1.13×10^{-2} mol l^{-1}; [NaBr] 0 (○), 2.63×10^{-3} (●), 5.25×10^{-3} (△), and 1.08×10^{-2} mol l^{-1} (▲); the inset shows the effect of NaBr on the observed rate constant at 2.5×10^{-3} mol l^{-1} CTAB (solid lines calculated; see text)

observed. A qualitative analysis of the mean degree of coverage (θ) for the CTA micelle by BP provides an insight into the most probable source of this discrepancy.

The mean degree of coverage (θ) can be defined as the number of bound BP per CTA micelle [equation (4)]. [BP_b]

$$\theta = [BP_b]/(C_D/\bar{n}) \quad (4)$$

was calculated,[1,7] and $\bar{n}$ can be taken as 100 under the experimental conditions used here. The value of θ was less than 10 up to [BP] = 2×10^{-4} mol l^{-1}, increasing to *ca.* 40 for the highest concentration of BP (Table). A micelle covered mainly with the bulky BP ions can behave in a manner quite distinct from that of a typical CTAB micelle, and thus the parameters used to calculate k_p (Table) could be different. Kinetic effects which depend on the nature of counterions have been clearly demonstrated in the case of spontaneous decarboxylation.[20] Thus the disagreement between the predicted and experimental values (Table) may result not from a failure of the model but from a change of a typical CTAB micelle to a BP-covered CTA micelle.

In conclusion, the analysis of the data presented in this work demonstrated that the effect of CTAB on the alkaline hydrolysis of BP can be treated quantitatively by using a pseudophase model with explicit consideration of ion exchange.* It is worth emphasizing that ionic substrates cannot be considered to be distributed between micelles and the aqueous phase like an uncharged solute.

Experimental

Materials.—Pure potassium BP was furnished by Fontoura-Whyeth Laboratories (São Paulo, Brazil). Stock solutions of BP were prepared in water and discarded after 2 h. CTAB (Merck) was recrystallised from ethanol–acetone.[8] Deionized, doubly distilled (glass) water was used throughout. All other reagents were of analytical grade.

Method.—Kinetic and spectral measurements were carried out at 30.0 °C with a Beckman M25 kinetic spectrophotometer. The alkaline hydrolysis of BP was followed by monotoring the disappearance of BP at 250 nm. Some measurements were performed at 238 nm, and in all cases the observed pseudo-first-order rate constants (k_ψ) were coincident with those obtained at 250 nm. All rate constants were calculated using a weighted least-squares program with an HP-90 calculator. The pseudo-first-order rate constants were obtained from at least three separate kinetic runs.

Acknowledgements

This work was supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), and Financiadora de Estudos e Projetos (FINEP). V. R. C. is a CAPES predoctoral fellow on leave from the Universidade Federal de Santa Catarina.

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Received 19*th June* 1984; *Paper* 4/1051