# Transition state stabilization by micelles: the hydrolysis of *p*-nitrophenyl alkanoates in cetyltrimethylammonium bromide micelles

**Oswald S. Tee and Alexei A. Fedortchenko**

**Abstract**: The cleavage of *p*-nitrophenyl alkanoates (acetate to octanoate) at high pH is modestly catalyzed by micelles formed from cetyltrimethylammonium bromide (CTAB) in aqueous solution. Rate constants exhibit saturation behaviour with respect to [CTAB], consistent with substrate binding in the micelles. The strength of substrate binding and transition state binding to the micelles increases monotonically with the acyl chain length, and with exactly the same sensitivity. As a result, the extent of acceleration (or catalytic ratio) is independent of the ester chain. These and earlier results are consistent with the reaction centre being located in the Stern layer of the micelle, with the acyl chain of the ester being directed into the hydrophobic micellar interior. The chain length dependence of kinetic parameters found in this work is comparable to that found previously for ester cleavage by cyclodextrins and by various enzymes with hydrophobic binding sites, as well as to that observed for other phenomena involving hydrophobic effects.

*Key words*: catalysis, ester hydrolysis, micelles, transition state.

**Résumé** : Le clivage des *p*-nitrophényl alcanoates (allant de l'acétate à l'octanoate) à pH élevé est modérément catalysé par des micelles formées à partir du bromure de cétyltriméthylammonium (BCTA) en solution aqueuse. Les constantes de vitesse exhibent un comportement de saturation par rapport à [BCTA], ce qui est en accord avec un substrat lié dans les micelles. La force de la liaison du substrat et la liaison de l'état de transition aux micelles augmente de façon monotone avec la longueur de la chaîne acyle, et avec exactement la même sensibilité. Conséquemment, l'importance du taux d'accélération (ou rapport catalytique) est indépendante de la chaîne de l'ester. Ces résultats ainsi que des résultats antérieurs sont en accord avec le centre de réaction localisé dans la couche de Stern de la micelle, avec la chaîne de l'ester dirigée vers l'intérieur hydrophobe de la micelle. L'influence de la longueur de la chaîne sur les paramètres cinétiques, mise en évidence dans ce travail, est comparable à celle trouvée antérieurement lors du clivage des esters par la cyclodextrine et par divers enzymes ayant des sites hydrophobes de liaison, aussi bien que celle observée pour d'autres phénomènes impliquant des effets hydrophobes.

*Mots clés* : catalyse, hydrolyse d'ester, micelles, état de transition.

[Traduit par la rédaction]

## Introduction

One of our current interests is the stabilization of transition states by catalysts (1). In this regard, we make use of an approach developed by Kurz for acid-catalyzed reactions (2), based on transition state theory. As detailed elsewhere (1–3), we define an apparent constant ($K_{TS}$) for dissociation of the transition state of the catalyzed reaction (TS.cat) into the transition state of the normal reaction (TS) and the catalyst (cat), as in eq. [1].

$$[1] \quad K_{TS} = \frac{[TS][cat]}{[TS.cat]} = \frac{k_u}{k_2} = \frac{k_u K_S}{k_c}$$

In eq. [1], $k_u$ is the rate constant in the absence of catalysis, and $k_2$ is the second-order rate constant for reaction with the catalyst. If saturation kinetics are observed, with a dissociation constant $K_S$ for the {substrate.catalyst} complex, and rate constant $k_c$ for its reaction, then $k_2 = k_c/K_S$. Note that according to eq. [1], $k_c/k_u = K_S/K_{TS}$, which implies that catalytic acceleration is a consequence of transition state binding that is stronger than substrate binding.

We have applied eq. [1] to many reactions mediated by cyclodextrins (1, 3–6) but it is equally applicable to reactions catalyzed by enzymes, catalytic antibodies, metal ions, acids, bases, and micelles (1). In fact, the approach has been used by enzymologists for many years (7), and it has recently been adopted by Kirby (8) for reactions catalyzed by enzyme mimics. In the present paper we again apply eq. [1] to a reaction that is catalyzed by micelles.

The effects of micelles on organic reactions have been extensively studied (9). These effects may be catalytic, or inhibitory, depending on the structure of the reactants, the charge type of the reaction, and the type of surfactant (cationic, anionic, or neutral) forming the micelles. For the most part, catalytic effects can be understood in terms of the ability of micelles to provide a new reaction environment, and to con-

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centrate reagents at the micelle–water interface, in the so-called Stern layer.

One of the classic papers in the field of micellar catalysis is that by Menger and Portnoy (10), who introduced a pseudophase model of catalysis. Among other things, they studied the basic hydrolysis of *p*-nitrophenyl acetate and *p*-nitrophenyl octanoate (pNPA and pNPO) in the presence of micelles formed from dodecyltrimethylammonium bromide. Previously, Cordes and co-workers (11) had looked at the effects of micelles on several reactions, including the hydrolysis of pNPA and *p*-nitrophenyl hexanoate (pNPH) in micelles formed from cetyltrimethylammonium bromide (CTAB). Shortly after, Romsted and Cordes (12) studied the effect of various *n*-alkyltrimethylammonium bromides on the cleavage of pNPH and *p*-nitrophenyl laurate (dodecanoate). The main conclusions of these early studies were that catalysis of the ester hydrolysis is greater for longer esters and longer surfactants, and that it is promoted by inclusion of the substrates in the micelles because hydroxide ions are concentrated in the Stern layer of cationic (but not anionic) micelles, near the reactive part of the ester.

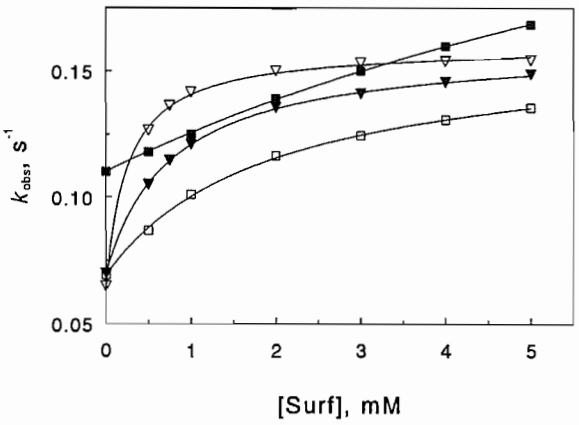
Later, Quina et al. (13) showed that the diminished rate of CTAB-catalyzed hydrolysis of pNPA and pNPO observed at high [CTAB] (>1 mM) can be explained by a preferential exchange of bromide ions for hydroxide ions in the Stern layer of the micelles. This and similar work on other micelle-catalyzed reactions gave rise to the pseudophase ionic exchange (PIE) model (14).

Much more recently, Al-Awadi and Williams (15) studied the hydrolysis of nine phenyl laurates (dodecanoates) in CTAB micelles. They found that the sensitivity of the micelle-catalyzed reaction to the leaving group ($\beta_{lg} = -0.51 \pm 0.06$) is virtually the same as that for hydrolysis in the basic aqueous medium ($\beta_{lg} = -0.56 \pm 0.05$). As we pointed out earlier (1), this situation arises because the binding of the esters and their transition states to the micelles is essentially independent of the substituent on the phenyl group. It also means that the transition state stabilization afforded by the CTAB micelles is the same regardless of the phenoxide leaving group, and it is consistent with the reaction centre being in a largely aqueous environment, while the acyl chain of the ester is directed into the hydrophobic interior of the micelle. It was suggested that this interpretation could be tested by studying a series of esters with different acyl chain lengths, to see if they show an appropriate sensitivity of transition state parameters to ester structure (1). The present paper describes just such a study.

## Results

We have measured the kinetics of hydrolysis of a series of *p*-nitrophenyl alkanoates (acetate to octanoate) at high pH (11.6, phosphate buffer) as a function of the concentration of cetyltrimethylammonium bromide (CTAB), which forms cationic micelles. Reactions were carried out with constant bromide ion concentration (= 5 mM) to avoid the problem of ion exchange in the Stern layer of the micelles (13–15). As expected from previous studies, the reaction is modestly catalyzed, and it exhibits saturation behaviour with respect to [CTAB], as shown by the examples presented in Fig. 1. Experiments with dodecyltrimethylammonium bromide (DDTB) were also attempted but they were not as successful, because the catalytic effect is small. This probably arises because the micelles of DDTB are smaller and the critical micellar concentration (cmc) of DDTB is higher (16), so that inclusion of the alkanoate esters (and their transition states) in the micelles is less favorable.

**Fig. 1.** Effect of cetyltrimethylammonium bromide (CTAB) on the rate of hydrolysis of *p*-nitrophenyl alkanoates: acetate, ■; butanoate, □; pentanoate, ▼; hexanoate, ▽.




Analysis of the kinetic data was based on the pseudophase model introduced by Menger and Portnoy (10). In this model, it is assumed that the organic substrate is distributed between the aqueous phase and the micellar phase, in accord with an apparent dissociation constant, $K_S$ (eq. [3]), and allowance is made for the hydrolysis of free ester (E) in the basic medium (eq. [2]) and of ester sequestered in the micellar pseudophase (E.Surf), eq. [3]. Together these two processes lead to a dependence of the overall rate constant for ester hydrolysis on concentration [Surf] as given in eq. [4].

$$[2] \quad \mathrm{E} \xrightarrow{k_u} \text{products}$$

$$[3] \quad \mathrm{E} + \mathrm{Surf} \underset{K_S}{\rightleftharpoons} \mathrm{E.Surf} \xrightarrow{k_c} \text{products}$$

$$[4] \quad k_{obs} = \frac{(k_u K_S + k_c[\mathrm{Surf}])}{(K_S + [\mathrm{Surf}])}$$

From experiments carried out over a range of [Surf] one can find values of $k_c$ and $K_S$ by nonlinear fitting of eq. [4], keeping $k_u$ fixed at the measured value. Often, the correction [Surf] = ([Surf]$_0$ − cmc) is made (9), where [Surf]$_0$ is the stoichiometric concentration of the surfactant. However, since the critical micellar concentration (cmc) of CTAB is $9.2 \times 10^{-4}$ M at zero ionic strength and much lower at high [salt] (16), we have followed Al-Awadi and Williams (15) and dispensed with the correction, in the interests of simplicity.[2] The measured values of $k_u$ and fitted values of $k_c$ and $K_S$ are collected in Table 1, which also contains values of $K_{TS}$, calculated from eq. [1].

[2] In fact, we have determined that use of the correction makes no significant difference to the fitted values of $k_c$ and $K_S$, or to the correlations of p$K_S$ and p$K_{TS}$ with chain length that are discussed below.

**Table 1.** Constants for the hydrolysis of *p*-nitrophenyl alkanoates in CTAB micelles, as a function of acyl chain length, *N*.[a]

| *N* | $k_u$, $s^{-1}$ | $K_S$, mM | $k_c$, $s^{-1}$ | $k_2$, $M^{-1}$ $s^{-1}$ | $K_{TS}$, mM |
|---|---|---|---|---|---|
| 2 | 0.110 | 11.4 ± 0.6 | 0.301 ± 0.008 | 26.4 | 4.17 |
| 3 | 0.107 | 5.29 ± 0.17 | 0.236 ± 0.003 | 44.6 | 2.39 |
| 4 | 0.0693 | 1.96 ± 0.10 | 0.161 ± 0.002 | 82.1 | 0.844 |
| 5 | 0.0697 | 0.769 ± 0.014 | 0.160 ± 0.001 | 208 | 0.335 |
| 6 | 0.0649 | 0.257 ± 0.013 | 0.160 ± 0.001 | 623 | 0.104 |
| 7 | 0.0630 | 0.0819 ± 0.0021 | 0.162 ± 0.001 | 1980 | 0.0319 |
| 8 | 0.0591 | 0.0423 ± 0.0031 | 0.147 ± 0.002 | 3480 | 0.0170 |

[a]At 25°C, in a 0.2 M aqueous phosphate buffer of pH 11.6 and with bromide ion concentration = [CTAB] + [NaBr] = 5.0 mM.

## Discussion

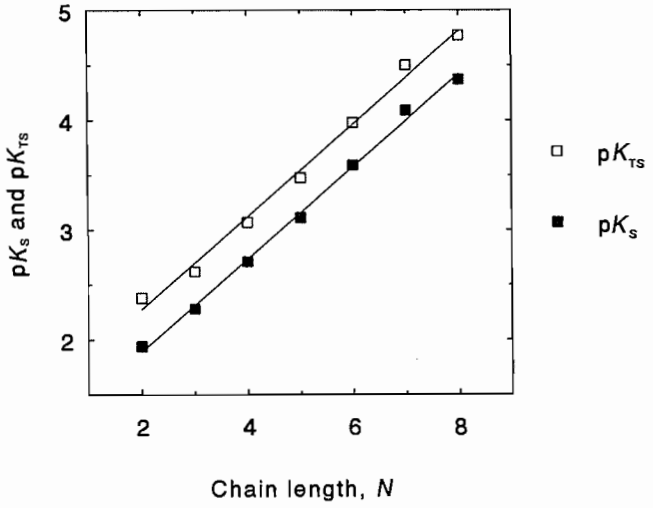
The constants in Table 1 can be used to probe the origins of the micellar catalysis of the hydrolysis of the esters. In particular, we can compare the sensitivities of substrate binding, reflected in $pK_S$ (= $-\log K_S$), and transition state binding, through $pK_{TS}$ (= $-\log K_{TS}$), to variations in the structure of the substrates (1).

From the data of Al-Awadi and Williams for phenyl laurate esters (15), both substrate and transition state binding parameters ($pK_S$ and $pK_{TS}$) are independent of the phenoxide leaving group (1). For the present results for *p*-nitrophenyl alkanoates, the plot of $pK_S$ against the ester chain length (*N*) is a straight line of slope 0.42 ± 0.01, and the plot of $pK_{TS}$ against N is parallel, with a slope of 0.42 ± 0.02 (Fig. 2). Moreover, the plot of $pK_{TS}$ against $pK_S$ is a good straight line ($r = 0.999$), and has a slope of 1.00 ± 0.01, implying that the variations in substrate binding and transition state binding are governed by the same factors.

According to eq. [1], the limiting acceleration, or catalytic ratio, $k_c/k_u = K_S/K_{TS}$. As a consequence, the parallelism between $pK_S$ and $pK_{TS}$, seen in Fig. 2, means that this ratio is essentially constant, at 2.4 ± 0.3, for acyl chain lengths from $C_2$ to $C_8$. So, contrary to what was surmised earlier from more limited data, the magnitude of the catalysis does not increase appreciably with the ester chain length, but the strength of substrate binding *and* transition binding does. Correspondingly, values of $k_2$ (= $k_c/K_S$) increase 130-fold, from 26 to 3480 $M^{-1}$ $s^{-1}$, under the reaction conditions. For the catalytic ratio $k_c/k_u$ to increase significantly with chain length would require the transition state binding ($pK_{TS}$) to be more sensitive to the acyl chain length than the initial state binding ($pK_S$).

The parallel dependence of $pK_S$ and $pK_{TS}$ on acyl chain length (Fig. 2) is consistent with incorporation of the substrates and their transition states in CTAB micelles being largely determined by hydrophobic effects. This assertion is based on the knowledge that many phenomena associated with hydrophobicity of *n*-alkyl derivatives vary linearly with chain length (16–21). For example, a plot of −log (cmc) against chain length (*N* = 10,12,14,16) for the micellization of *n*-alkyltrimethylammonium bromides is a straight line ($r$ = 0.9998), with slope of 0.313 ± 0.004 (from data in Rosen (16)); other *n*-alkyl ionic surfactants show comparable behaviour (16, 17). Also, free energies of transfer (water → micelle, water → organic medium, and water → gas phase) for organic solutes vary linearly with solute chain length, and the solubility of organic solutes in micelles increases exponentially with surfactant chain length (16–19). In a similar way, the Hansch hydrophobicity parameters ($\pi$), which are based on partition coefficients, are also linear in *N* (20), as are microhydrophobicity parameters derived by Menger from kinetic measurements (21).

**Fig. 2.** Dependence of transition state binding ($pK_{TS}$, □) and substrate binding ($pK_S$, ■) to CTAB micelles on the acyl chain length (*N*) of *p*-nitrophenyl alkanoate esters. The slopes of both graphs are 0.42 ± 0.02. The corresponding plot of $pK_{TS}$ against $pK_S$ is linear ($r$ = 0.999), and has a slope of 1.00 ± 0.01, suggesting that the same factors affect transition state binding and substrate binding.




In other work, we have shown that the strength of binding of many *n*-alkyl derivatives to cyclodextrins (22) is sensitive to chain length, with slopes of $pK_S$ vs. *N* generally falling in the range 0.3–0.6 (23). Moreover, $pK_{TS}$ values for the basic cleavage of *p*-nitrophenyl alkanoates by cyclodextrins vary linearly with *N*, with slopes of 0.2–0.3, except for short acyl chains (4*a*, 4*c*, 4*d*). These sensitivities to chain length are due, at least in part to hydrophobic effects (4*e*).

Another interesting comparison, derived from observations made by O'Connor and co-workers (24), is with the cleavage of esters, including *p*-nitrophenyl alkanoates, by various enzymes (lipases, chymotrypsin, trypsin, horse liver esterase),

which also shows distinct chain length dependences, with plots of log ($k_{obs}$) vs. $N$ having slopes of 0.3–0.4. These values are consistent with the acyl binding site of the enzymes being quite hydrophobic. Similarly, values of log ($k_{cat}/K_M$), and hence $pK_{TS}$, for the cleavage of methyl esters of *N*-acetyl-L-α-amino acids by α-chymotrypsin correlate strongly with the Hansch hydrophobicity parameters of the α-substituents (1, 25).

It seems reasonable to conclude from all these observations that stabilization of the transition states in the various cases are partially or largely governed by similar hydrophobic effects.

## Conclusions

In his recent discussions of the "molecular recognition of transition states," Kirby (8, 26) has emphasized two distinct components to transition state stabilization: *passive binding*, which arises from non-covalent interactions, comparable to those involved in equilibrium binding; *dynamic binding*, due to interactions at the reaction centre, particularly those associated with the making and breaking of covalent bonds. Seen in these terms, our conclusions for the hydrolysis of phenyl alkanoates in CTAB micelles are as follows: (*a*) the passive binding of the transition state involves hydrophobic binding of the acyl chain of the esters in the micelles, as does the substrate binding (Fig. 2); (*b*) the dynamic component is small and essentially constant, arising from the acyl transfer taking place in the largely aqueous environment of the Stern layer (1, 15).

## Experimental

The esters were purchased from Sigma or were available from previous studies (4*a*, 4*c*, 4*d*, 4*e*). Cetyltrimethylammonium bromide (hexadecyltrimethylammonium bromide, CTAB) was obtained from ICN Biochemicals. All other chemicals were from the Aldrich Chemical Company, except for standard NaOH solutions, which were obtained from A & C Chemicals (Montreal).

The kinetics of the ester cleavage were followed by monitoring the first-order appearance of the *p*-nitrophenolate ion at 405 nm, using an Applied Photophysics SX17MV stopped-flow spectrophotometer, with the temperature of the cell kept at 25.0 ± 0.1°C. Usually, about 5–10 absorbance traces were computer averaged before estimation of $k_{obsd}$ by nonlinear least-squares fitting of an exponential growth curve, using software supplied with the instrument.

Substrate concentrations were kept low with respect to [CTAB] so that the properties of the micelles were not significantly perturbed by the presence of the substrate (9, 10). Stock ester solutions (0.1 M in acetonitrile) were diluted in water to give twice the desired final concentrations. These were mixed 1:1 with a solution containing 0.4 M phosphate buffer + CTAB + NaBr whose pH had been adjusted to 11.6. Both reactant solutions were sonicated for 10–20 min to facilitate solubilization. The final reacting solutions contained 0.2 M phosphate buffer, CTAB (0–5 mM), NaBr (5 mM − [CTAB]), and ester concentrations as follows: acetate to pentanoate, 50 μM; hexanoate, 25 μM; heptanoate, 5 μM; octanoate, 2.5 μM.

Nonlinear fitting of eq. [4], and other such equations, was carried out with commercial (Prism) software.

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