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MECHANISM OF MICELLAR EFFECTS IN IMIDAZOLE CATALYSIS

ACYLATION OF BENZIMIDAZOLE AND ITS N-METHYL DERIVATIVE BY p-NITROPHENYL CARBOXYLATES

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Abstract—The effect of sodium dodecylsulphate (SDS) and cetyltrimethylammonium bromide (CTAB) on the rate of acylation of benzimidazole and its N-Me derivative by *p*-nitrophenyl carboxylates over a wide pH range (5–11·5) has been studied. (i) Both cationic and anionic micelles produce but a weak effect on the acylation of both N-methylbenzimidazole and the electroneutral form of benzimidazole. The fact that no micellar effects seem to be present is accounted for by that the favourable contribution due to increasing the reagent concentration in the micellar "phase" (~100-fold acceleration in the case of *p*-nitrophenyl heptanoate) is almost completely compensated by the unfavourable effect of the micellar environment on the true second-order rate-constant. (ii) CTAB micelles are extremely effective catalysts of acylation of benzimidazole anion. The mechanism of acceleration (~ 10^5 times in the case of *p*-nitrophenyl heptanoate) is due to the reagents being concentrated in the micelles (~100 times), to the apparent pK_a shift of the nucleophile under the action of the surface micelle charge (~100 times) and to the favourable effect of the micellar environment on the true second-order rate-constant (~10 times). (iii) The inhibiting effect of salts ($F^- < Cl^- < BrO_3^- < Br^- < NO_3^-$) on micellar catalysis (a 100-fold inhibition in the presence of 0·12 M KNO_3) has only one cause—a lower solubility of the anionic reagent by the cationic micelles. (iv) Comparison is made of the true reactivity of electroneutral (AH) and anionic (A^-) forms of benzimidazole in the surface layer of the micelle. If in water A^- exceeds AH by approx 10^3 times with respect to nucleophility, in the micellar environment the ratio of the true second order rate-constants is as high as 10^6. A mechanism of this phenomenon is suggested, which may also help understand certain polymer and protein effects on the imidazole catalysis.

INTRODUCTION

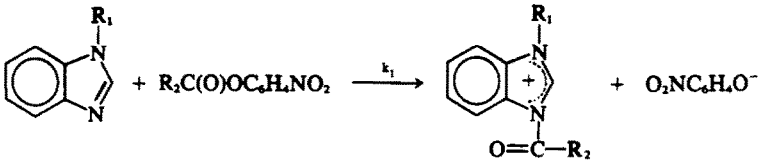
Studies of micellar effects have a series of important aspects.[1,2] The present work deals with the micellar effects in the reactions of imidazole. The choice of this subject was due to the fact that the mechanism of the imidazole-catalysed reactions is one of the key problems of homogeneous catalysis, which is still far from being solved.[3,4] Moreover, bioorganic chemistry is also interested in imidazole catalysis, as the imidazole group is a part of the active centre of many enzymes.[3-5]

We have studied the effect of ionogenic surfactant micelles on acylation of benzimidazole and its N-methyl derivative by *p*-nitrophenyl carboxylates (Scheme 1).† A wide pH range has been employed, so that the reaction involving benzimidazole anion could be studied (Schemes 2–3).

The micellar effects in the hydrolysis of *p*-nitrophenyl esters catalysed by imidazole and its derivatives have long attracted attention.[7-10] This study differs from the previous ones: Firstly, the pH-dependent micellar catalysis in our system (Schemes 1–3) is extremely efficient. For example, acylation of benzimidazole by *p*-nitrophenyl heptanoate accelerates up to 10^5 times under the action of cationic CTAB‡ micelles. Secondly, we have revealed the unusual phenomenon that micellar catalysis is substrate specific. For example, if only one N-Me group is incorporated into a molecule of ben-

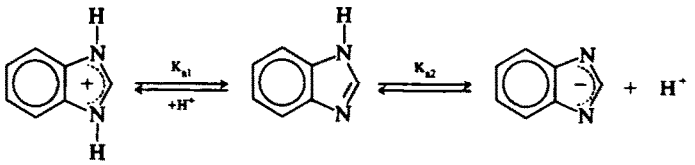
†See preliminary communication.[6]

‡Abbreviations used: CTAB, cetyltrimethylammonium bromide; SDS, sodium dodecylsulphate; CMC, critical micelle concentration.

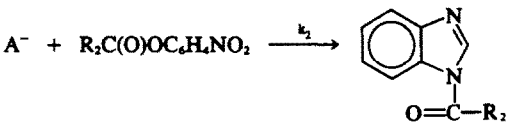


where $R_1 = H, CH_3$ and $R_2 = CH_3, CH_3(CH_2)_2, CH_3(CH_2)_5$

SCHEME 1



SCHEME 2



SCHEME 3

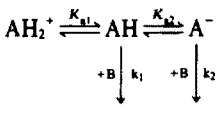
zimidazole (which does not noticeably alter the reaction rate in water), the effective reactivity of the nucleophile in the presence of CTAB micelles decreases by more than 10^5 times. The third aspect, is that the results of our experiments have been quantitatively evaluated in terms of the recently developed kinetic theory of micellar catalysis.[2,11-14]

This investigation has resulted in a general mechanism for the micellar effects on imidazole catalysis in hydrolysis of *p*-nitrophenyl carboxylates (Discussion). In terms of this mechanism, some aspects of the polymer and protein which have previously been reported (Conclusion) may be explained.

THEORY

pH and Surfactant concentration effects on the rate of bimolecular reaction with an ionogenic reagent

Let us consider the kinetics of the bimolecular reaction in which one of the reagents exists in any of three forms, i.e. as cation AH_2^+, an electroneutral compound AH and anion A^-:



(1)

assuming that the overall rate of the bimolecular reaction,

$$v = k_1[AH][B]_t + k_2[A^-][B]_t,$$

is only determined by the reactions involving the AH and A^- forms. In this case, for the apparent rate constant, which is $k_{app} = v[A]_t[B]_t$, the following pH-dependent expression is valid:

$$k_{app} = \frac{k_1}{1 + \frac{[H^+]}{K_{a1}} + \frac{K_{a2}}{[H^+]}} + \frac{k_2}{1 + \frac{[H^+]}{K_{a2}} + \frac{[H^+]^2}{K_{a1}K_{a2}}} \quad (2)$$

†See the Refs in review.[2]

where k_1 and k_2 are the second order rate constants; K_{a1} and K_{a2} are dissociation constants; $[A]_t = [AH_2^+] + [AH] + [A^-]$ and $[B]_t$ are the total concentrations of the reagents.

When considering the kinetics of reaction (1) which proceeds in the presence of the surfactant, one should take into consideration that the reagents are distributed between the water and the micelles.[13] This means that the overall rate of the reaction should be regarded as the sum of the rates in the aqueous and micellar "phases", as was previously suggested.[13] The problem becomes much simpler if one assumes that the equilibrium of reagents is promptly achieved and is not disturbed in the course of the chemical reaction.† In this case for the apparent rate constant, equation (2) is still valid, the only difference being that its constants are now the functions of the surfactant concentration:

$$k_{1,app} = \frac{(k_{1,m}/v)K_{AH}K_BC + k_{1,b}}{(1 + K_{AH}C)(1 + K_BC)} \quad (3a)$$

$$k_{2,app} = \frac{(k_{2,m}/V)K_{A^-}K_BC + k_{2,b}}{(1 + K_{A^-}C)(1 + K_BC)} \quad (3b)$$

$$K_{a1,app} = K_{a1,b}\frac{1 + K_{AH}C}{1 + K_{AH_2^+}C} \quad (4a)$$

$$K_{a2,app} = K_{a2,b}\frac{1 + K_{A^-}C}{1 + K_{AH}C} \quad (4b)$$

where C is concentration of the surfactant from which CMC is subtracted; V is molar volume of the surfactant; indices *m* and *b* show that a value (for example, a rate or dissociation constant) belongs to the micellar or bulk phases, respectively. The binding constants should be determined by the following ratios (see[15]):

$$K_{AH} = (P_{AH} - 1)V, \quad K_{AH_2^+} = (P_{AH_2^+} - 1)V,$$
$$K_{A^-} = (P_{A^-} - 1)V, \quad K_B = (P_B - 1)V, \quad (5)$$

where P are the partition coefficients for the respective

reaction components which are equal to $P_{AH} = [AH]_m/[AH]_b$, $P_{AH_2^+} = [AH_2^+]_m/[AH_2^+]_b$, etc.

It should be added that Eqs (3) are valid only if $P \gg 1$ (i.e. the reagents firmly bind to the micelles) and $CV \ll 1$ (i.e. the volume fraction of the micellar phase is small); see[2,13] for details.

The theoretical dependence of the apparent rate constant, k_{app}, on the surfactant concentration and pH may be found if Eqs (3) and (4) are combined with expression (2). Of course, in the general case we shall have a cumbersome formula; but for the purpose of this work consideration of some particular case will be sufficient and this will be done below.

RESULTS

Specificity towards the nucleophile. It is well known that imidazole and N-methylimidazole hardly differ in the acylation rate by *p*-nitrophenyl carboxylates.[4] As is seen from the data obtained by us (Fig 1, see data without the surfactant) benzimidazole and its N-Me derivative display similar reactivities (at pH 8). It was interesting to establish to what degree the micellar effects are sensitive to the nature of these nucleophiles with equal reactivities in water. Such an experiment was run at pH 8–8·8, when benzimidazole exists in only one, electroneutral, form (*cf* the data on the effect of the surfactants on the apparent pK_a values of benzimidazole[16]).

The anionic SDS micelles produce a rather weak effect on acylation of both benzimidazole (3-fold acceleration) and its N-Me derivative (a 2-fold deceleration) (see Fig 1).

The kinetic regularities which manifest themselves in the presence of CTAB are of much greater interest. As is seen in Fig 2, the effect of cationic micelles on the reaction proved to be different with different nucleophiles. In the case of benzimidazole (curve **a**), the micellar catalysis is extremely effective. The acceleration

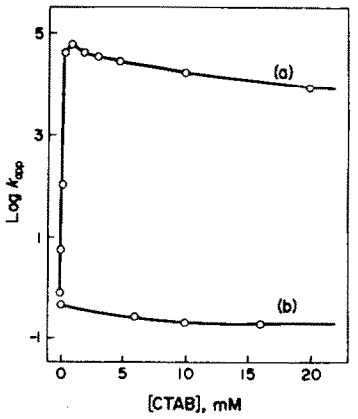


Fig 2. Dependence of the logarithm of the apparent rate constant ($M^{-1}\,min^{-1}$) on the CTAB concentration in acylation of benzimidazole (**a**) and N-methylbenzimidazole (**b**) by *p*-nitrophenyl heptanoate. Experimental conditions: 30°; pH 8·8 (**a**) and pH 8 (**b**); 0·02 M borate buffer; 1 vol % of dimethylsulfoxide.

observed (~10^5-fold) is 2–3 orders higher than the micellar effects previously obtained with imidazole.[7,8,10] On the other hand, incorporation of just one N-Me group into the nucleophile molecule completely eliminates the favourable effect of the CTAB micelles on acylation (curve **b**).

Specificity towards ester. The micellar effects in acylation of benzimidazole by *p*-nitrophenyl carboxylates depend to a slight extent on the length of the aliphatic chain in the ester molecule (Fig 3). For example, the maximal acceleration value $(k_{app})_{max}/(k_{app})_{C=O}$ changes in the acetate–heptanoate series only 12-fold (Table 1). This does not agree with the 100-fold increase reported for the same series by Gitler and Ochoa-Solano[8] for acylation of imidazole covalently bound to the micelle.[1] However, as

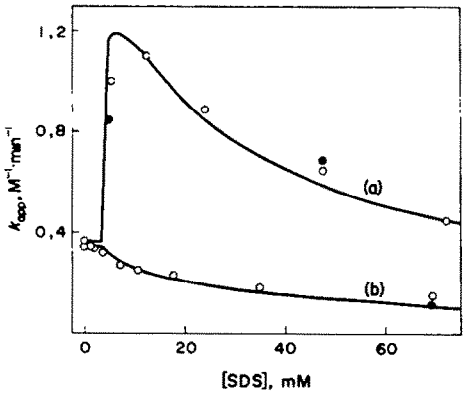


Fig 1. Dependence of the apparent rate constant on the SDS concentration for acylation of benzimidazole (**a**) and N-methylbenzimidazole (**b**) by *p*-nitrophenyl heptanoate. Black circles—in the presence of 0·1 M NaCl. The curves are theoretical and correspond to Eq (8) with the use of the values of the rate and binding constants given in Tables 2 and 3. Experimental conditions: 30°; pH 8; 0·02 M borate buffer; 1 vol % of dimethylsulfoxide.

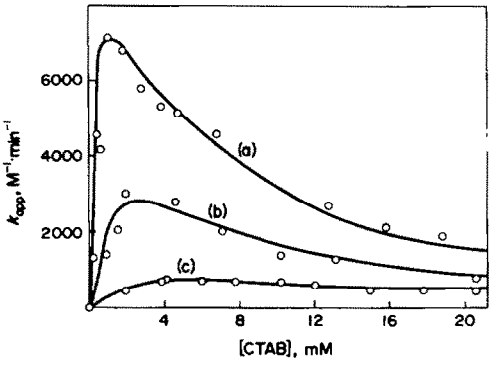


Fig 3. Dependence of the apparent rate constant on the CTAB concentration in acylation of benzimidazole by *p*-nitrophenyl heptanoate (**a**), *p*-nitrophenyl butyrate (**b**) and *p*-nitrophenyl acetate (**c**). The curves are theoretical and correspond to Eq (11) with the use of the values of the rate constant $k_{2,m}/V$ and of the binding constants given in Tables 1 and 3. The values of the rate constants of acylation by heptanoate, butyrate and acetate in the absence of CTAB are equal to 0·37, 0·45 and 0·57 $M^{-1}\,min^{-1}$, respectively. Experimental conditions: 30°; pH 8·2; 0·02 M borate buffer; 1 vol % of dimethylsulfoxide.

Table 1. Acylation of benzimidazole anion by *p*-nitrophenyl carboxylates in the presence of CTAB micelles. Conditions: 30°; 0·02 M borate buffer, 1 vol % of dimethylsulfoxide

Rate constants / *p*-nitrophenyl esters	$k_{2,b}$ M^{-1} min^{-1}	$(k_{app})_{max}/(k_{app})_{C=O}$	$k_{2,m}/V$ min^{-1}	$k_{2,m}/k_{2,b}$
Acetate	1260[a]	1540[b]	32400[b,e,f]	9·5[h]
Butyrate	710[a]	6700[b]	21000[b,e,f]	11[h]
Heptanoate	850[a]	19200[b]	32400[b,e,f]	14·1[h]
		80000[c]	32900[c,e,f]	14·3[h]
		93[d]	25700[d,e,g]	11·2[h]

[a] The pH-independent rate-constant of acylation of benzimidazole anion without a surfactant at $pH > pK_{a2,b}$. In Fig 4 the data on heptanoate, curve **a**, are given as an example.
[b] From the data in Fig 3 (pH 8·2).
[c] From the data in Fig 2, curve **a** (pH 8·8).
[d] In the presence of 0·12 M KNO_3; from the data in Fig 5, curve c (pH 7·7).
[e] The pH-independent value.
[f] Calculated with the use of Eq (11), see Appendix, Approach II.
[g] Calculated with the use of Eq (13).
[h] Calculated from the data for $k_{2,b}$ and $k_{2,m}/V$ (measured experimentally, see the same Table), with the use of $V = 0{\cdot}37\ M^{-1}$, see[17].

will be shown in the Discussion, this discrepancy may be very convincingly explained in terms of the kinetic theory of micellar catalysis.

pH-Dependence. The pH-dependence of benzimidazole acylation by *p*-nitrophenyl heptanoate has been studied in greater detail. The "rate-pH" profile of this reaction which proceeds in water (without the surfactant, see Fig 4, curve **a**) agrees qualitatively with the data of Bruice *et al.* on acylation of imidazole derivatives by *p*-nitrophenyl acetate.[4,18] For example, curve *a* shows that the reaction rate depends on both pK_a values for benzimidazole (Scheme 2); and the inflexion at a pH 8·8 is clearly due to the fact that the reactivity of benzimidazole anion is much (by more than 3 orders) higher than that of the electroneutral nucleophile.

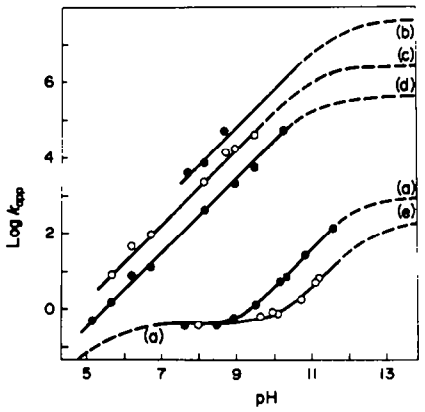


Fig 4. pH-dependence of the logarithm of the apparent rate constant (M^{-1} min^{-1}) on the CTAB concentration in acylation of benzimidazole by *p*-nitrophenyl heptanoate; (**a**) no surfactant; (**b**) 1 mM CTAB (optimal concentration of the surfactant); (**c**) 10 mM CTAB; (**d**) 89 mM CTAB; (**e**) 100 mM SDS; (■) 0·12 M KNO_3, no surfactant. The broken curves are theoretical with the $pK_{a,app}$ values given in[16]. Experimental conditions: 30°; 0·02 M borate buffer ($pH > 7{\cdot}5$) or 0·02 M phosphate buffer ($pH < 7{\cdot}5$); 1 vol % of dimethylsulfoxide.

The "reaction rate-pH" profile in the presence of SDS micelles is of the same character. As is seen in Fig 4 (curve **e**), anionic micelles hardly produce any affect on the behaviour of the electroneutral form of benzimidazole. The inhibiting effect observed at higher pH values is mainly due to the fact that in the presence of SDS micelles the fraction of the anionic form of benzimidazole, which has a high reactivity, decreases at the expense of the apparent pK_{a2} shift towards more alkaline pH range (*cf* data on the effect of the surfactants on the apparent pK_a values of benzimidazole[16]).

With CTAB micelles the situation is quite different. As is seen in Fig 4 (curves **b-c-d**), over the whole pH range studied, the reaction markedly accelerates, the "log k_{app} – pH" profile being a straight line with the slope equal to unity.

So, the pH-dependent micellar effects in the benzimidazole acylation is extremely sensitive to the nature of the surfactant. The effects are most striking in the pH 9·5–11·5 range, where the $\sim 10^5$-fold acceleration observed in the presence of the CTAB micelles gives way to a ~10-fold deceleration in the presence of SDS (Fig 4).

Salt effects. The reaction rate in the absence of the surfactant hardly depends on the ionic strength of the solution either in acylation of the electroneutral benzimidazole (pH 7·7, Table 2) or with benzimidazole anion (pH 10·3, Fig 4). At the same time, the micellar catalysis observed in the presence of CTAB micelles is strongly inhibited by added electrolytes (counter ions; Fig 5). For

Table 2. Acylation of electroneutral benzimidazole and N-methylbenzimidazole by *p*-nitrophenyl heptanoate in the presence of CTAB and SDS micelles. Conditions: 30°, 0·02 M borate buffer; 1 vol % of dimethylsulfoxide

Reaction system \ Rate constants	$k_{1,b}$ M^{-1} min^{-1}	$(k_{app})_{max}/(k_{app})_{C=O}$	$k_{1,m}/V$ min^{-1}	$k_{1,m}/k_{1,b}$
Benzimidazole + SDS	0·37[a]	3[b]	0·048[b,d]	0·033[f]
N-methylbenzimidazole + CTAB	0·35	3-fold deceleration[c]	0·0086[c,e]	0·009[f]
N-methylbenzimidazole + SDS	0·35	2-3-fold deceleration[b]	0·01[b,e]	0·007[f]

[a] In the presence of 0·12 M KNO_3 or KCl the values determined are 0·44 or 0·42 M^{-1} min^{-1}, respectively.
[b] From the data in Fig 1.
[c] From the data in Fig 2, curve b.
[d] Calculated with the use of Eq (8); see Appendix, Approach I.
[e] Calculated with the use of Eq (8) and the values of binding constants indicated in Table 3.
[f] Calculated from the data for $k_{1,b}$ and $k_{1,m}/V$ (experimentally measured; see the same Table 2), with the use of $V = 0·37\ M^{-1}$ for CTAB and $V = 0·25\ M^{-1}$ for SDS.[17]

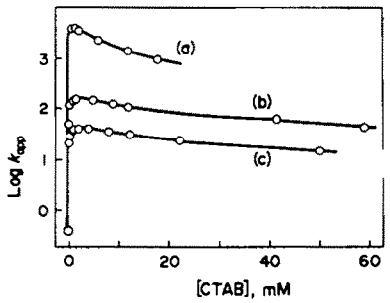


Fig 5. Dependence of the logarithm of the apparent rate constant (M^{-1} min^{-1}) on the CTAB concentration in acylation of benzimidazole by *p*-nitrophenyl heptanoate in the absence of salt (a), in the presence of 0·12 M KCl (b), in the presence of 0·12 M KNO_3 (c). Curves a and c are theoretical, correspond to Eq (11) with the values of $k_{2,m}/V$ and binding (K_{AH}, K_{A^-}, K_B) constants given in Tables 1 and 3. Experimental conditions: 30°; pH 7·7; 0·02 M borate buffer; 1 vol % of dimethylsulfoxide.

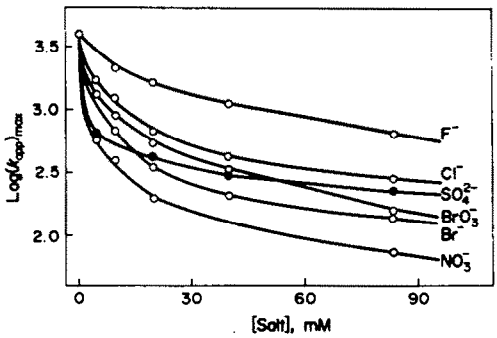


Fig 6. Dependence of the logarithm of the apparent rate constant (M^{-1} min^{-1}) on the concentration of added salts in acylation of benzimidazole by *p*-nitrophenyl heptanoate. Experimental conditions: 30°; pH 7·7; 0·02 borate buffer; 1 vol % of dimethylsulfoxide; [CTAB] = 1 mM.

example, the inhibiting effect of 0·12 M KNO_3 may be as high as two orders of magnitude, compare curves a and c.

As is seen in Fig 6, the monovalent anions are in the following sequence with respect to the effectivity of their inhibiting action: $F^- < Cl^- < BrO_3^- < Br^- < NO_3^-$. In the reactions involving enzymes[19] and also in ionic reactions in which the micelles of ionogenic surfactants take part,[20-23] anions make an analogous sequence with respect to their inhibiting capacity.

DISCUSSION

When studying the reasons why the reaction rate changes under the action of surfactant micelles, one should analyse separately the following two aspects: first, how increasing the concentration of the reagents in the micelles contributes to the acceleration of the reaction; and how the reactivity of the reaction components changes in a micellar environment in comparison with water. To answer these questions let us consider the experimental results in terms of the recently developed kinetic theory of micellar catalysis.[2,11-14] The theory makes it possible to find both the constants of binding of the reagents with micelles and the *true* rate-constant in the micellar "phase", based on the overall kinetic data, i.e. from experimentally established "reaction rate-surfactant concentration" profiles (Figs 1–3). To solve this problem, the approaches described in the Appendix are applicable. The resulting "elementary" rate and binding constants are presented in Tables 1–3.

Kinetic micellar effects in acylation of electroneutral form of benzimidazole and N-methylbenzimidazole

(i) *Evaluation.* A kinetic study of acylation of benzimidazole was performed over a wide, although limited, pH range, i.e. $pK_{a1,app} < pH < pK_{a2,app}$ (Fig 4). It seems that under the conditions employed a reaction involving the electroneutral AH form (Eq 1) may only be detected if anion A^- has a reactivity which does not exceed much that of AH, namely if

$$k_{2,app}/k_{1,app} \ll K_{a1,app}/K_{a2,app}. \quad (6)$$

In this case, the *pH-independent* overall rate of the process (measured experimentally in the range of

Table 3. Binding constants, K (l/mole) characterizing the incorporation of the reagents into the CTAB and SDS micelles (Eq 5). Conditions: 30°, 0·02 M borate buffer; 1 vol % of dimethylsulfoxide

Surfactants \ Reagents	N-methyl-benzimidazole	Benzimidazole cation (AH_2^+)	Benzimidazole electroneutral form (AH)	Benzimidazole anion (A^-)	*p*-Nitrophenyl acetate	*p*-Nitrophenyl butyrate	*p*-Nitrophenyl heptanoate
CTAB	34[a]	<1[b]	33[a] 37[b]	4000–5000[c] 50[d,e]	27[f]	530[g]	3800[d,e] 3600[g,e] 3000[g]
SDS	30[a]	2400[b]	28[a] 30[b] 30[h]	—	—	—	1500[g] 2000[h]

[a] From the dependence of the difference spectrum of the reagent on the surfactant concentration.[16]
[b] From the dependence of the apparent pK_{a1} value on the surfactant concentration.[16]
[c] At [CTAB] → CMC. Found from the dependence of the apparent pK_{a2} value on the CTAB concentration.[16] The dependence of K_A^- on CTAB concentration is given in[16].
[d] From the dependence of the apparent rate constant on the CTAB concentration (Fig. 5, curve c), with the use of Eq (13).
[e] In the presence of 0·12 M
[f] Found by gel filtration.[22]
[g] From the dependence of the solubility of the reagent on the surfactant concentration.[13]
[h] From the dependence of the apparent rate constant, k_{app}, on the SDS concentration (Fig 1), with the use of Eq (8); see Appendix, Approach I.

$pK_{a1,app} < pH < p(K_{a2,app}k_{2,app}/k_{1,app})$ is determined entirely by the acylation of the electroneutral form of benzimidazole, i.e.

$$k_{app} \approx k_{1,app}. \tag{7}$$

This conclusion follows from an analysis of Eq (2).

As is obvious in Fig 4, requirement (6) which holds when there is no surfactant (curve **a**), is also true in the presence of SDS micelles (curve **e**). Therefore, based on Eqs (3) and (7), the apparent value of the pH-independent rate-constant, k_{app} may be presented as:

$$k_{app} = \frac{(k_{1,m}/V)K_{AH}K_BC + k_{1,b}}{(1 + K_{AH}C)(1 + K_BC)}. \tag{8}$$

We have analysed the experimental data (i.e. the "k_{app}-C" profile; Fig 1, curve **a**) in terms of Eq (8); see Approach I in Appendix. As a result, we have found both the effective value of the true rate-constant in the micellar medium ($k_{1,m}/V$; Table 2) and binding constants K_{AH} and K_B which characterize the incorporation of the two reagents (benzimidazole and *p*-nitrophenyl heptanoate) into the SDS micelles. As is obvious from Table 3, the values of the binding constants thus obtained agree with the values reported by other methods. This shows that the kinetic theory, Eqs (2–4) and, in particular, Eq (8), describes the experimental results correctly.

(ii) *Mechanism.* Let us analyse the physico-chemical reasons why the reaction is accelerated in the presence of SDS micelles. The value of maximal acceleration may be presented, based on (8), as follows (see also[13]):

$$\frac{(k_{app})_{max}}{(k_{app})_{C=0}} = \frac{k_{1,m}}{k_{1,b}} \cdot \frac{K_{AH}K_B}{V(\sqrt{(K_{AH})} + \sqrt{(K_B)})^2} \tag{9}$$

provided that $k_{1,app} \gg k_{1,b}$. It is seen in Table 3 that both the electroneutral benzimidazole and *p*-nitrophenyl heptanoate firmly bind with the micelles ($K_{AH} \gg 0$, $K_B \gg 0$). According to Eq (9), this should have resulted in a considerable (~100 times) acceleration of the reaction, if one assumes that V = 0·25 l/mole.[17] However, in the experiment, the pH-independent rate of benzimidazole acylation by *p*-nitrophenyl heptanoate increases insignificantly in the presence of SDS micelles (Fig 1, curve **a**). Such apparent absence of micellar effect is due to the fact that when the reaction is carried out in a micellar medium instead of water, the true value of second order rate constant markedly decreases, i.e. $k_{1,m} \ll k_{1,b}$ (Table 2). As a result, the favourable contribution by increasing the concentration of the reagents in the micelles makes to the acceleration of the reaction is almost fully compensated by the unfavourable effect of the micellar environment.

A similar explanation may be offered for the absence of micellar effects in acylation of N-methylbenzimidazole (Figs 1 and 2, curves **b**). Here too, the binding between the reagents and micelles is rather firm (Table 3). However, the considerable increase in the concentration of the reagents in the micelles is compensated by the unfavourable effect of the micellar environment on the reaction. One may see in Table 2 that the values of the true rate constant in both CTAB and SDS micelles are almost 100 times as low as that in water.

The fact that the true rate constant of acylation of the electroneutral form of benzimidazole (or N-methylbenzimidazole) by *p*-nitrophenyl carboxylates sharply decreases when the reaction is transferred from water to a micellar medium may be due to the fact that the transient state is more polar than the starting compounds (possibly, because the transient state is close to a tetrahedral complex with delocalized charges[3,4]). If this is the case,

the reaction rate should decrease as does the dielectric permeability of the medium or its solvation capacity.[24,25] These very properties are inherent in the micellar medium. For example, the dielectric permeability value in the micelles is much lower than in water not only inside the micelles but also in the surface layer.[26] The content of water in the micelle sharply decreases from the surface layer to the hydrophobic nucleus.[27] The results of the model experiment also show that the above supposed reasons for the "inhibiting action" of the micellar medium are correct. It was found earlier[6] that an increase in the concentration of the organic component in a water–ethanol mixture induces a considerable deceleration in the acylation of imidazole derivatives by *p*-nitrophenyl carboxylates.

The mechanism of the kinetic micellar effect in acylation of benzimidazole anion

(i) *Evaluation.* In contrast to the data obtained without surfactants (Fig 4, curve **a**) the "k_{app}-pH" profile observed in the presence of CTAB micelles (Fig 4, curves **b–d**) is a straight line with the slope being equal to unity over the whole pH range studied. This result, if analysed in terms of Eq (2) allows the conclusion that, in the presence of CTAB micelles, the overall kinetics of reaction (1) is determined entirely by the acylation of benzimidazole anion. Hence the following equation may be written for the true second order rate constant:

$$k_{app} \approx k_{2,app}\frac{K_{a2,app}}{[H^+]} \qquad (10)$$

which follows from (2) if $pK_{a1,app} < pH < pK_{a2,app}$ on the assumption that $k_{1,app}$ is sufficiently low. Substituting (3) and (4) into Eq (10), we have:

$$k_{app} = \frac{(k_{2,m}/V)K_A^- K_B C}{(1+K_{AH}C)(1+K_B C)} \cdot \frac{K_{a2,b}}{[H^+]} \qquad (11)$$

if one bears in mind the experimental fact (Fig 4) that in the presence of CTAB the acceleration of the reaction is so high that the reaction rate in water may be neglected.

We have analysed the experimental results (i.e. the "k_{app}-C" profile, Fig 2, curve **a**) in terms of Eq (11). The only problem in the analysis is that the value of K_A^- is not constant; it decreases somewhat as the CTAB concentration increases, which was demonstrated previously in the study of the surfactant effect on the apparent pK_{a2} value of benzimidazole.[16] Therefore, from the kinetic data one may determine only the true-rate constant in the micellar environment; see Approach II in Appendix. The $k_{2,m}/V$ values are listed in Table 1.

(ii) *Maximal acceleration of acylation of benzimidazole anion.* Let us make a quantitative analysis of the physico-chemical reasons determining such a considerable acceleration of the anionic reaction observed in the presence of CTAB micelles. As is seen in Fig 4, a value of acceleration equal to $k_{app}/(k_{app})_{C=O}$ is pH-dependent. The micellar effect reaches a maximum in the pH region where the two "k_{app}-pH" profiles compared (the first in the presence of CTAB micelles, for example, curve **b**, and the other at C=O, curve **a**) are straight lines with the slope equal to unity. In this optimal range $p(K_{a2,b}k_{2,b}/k_{1,b}) < pH < pK_{a2,app}$, the value of the micellar effect, although being pH-independent, changes with the concentration of the surfactant (compare curves **b–d** in Fig 4). That an optimal surfactant-concentration exists is clearly seen in Fig 2 (curve a), where the "k_{app}-C" profile at pH 8·8 is shown. According to theory,[2,11–14] using equations (10) and (11), the following equation for the pH-independent maximal acceleration corresponding to the optimal concentration of the surfactant (which is $1/\sqrt{[K_{AH}K_B]}$):

$$\frac{(k_{app})_{max}}{(k_{app})_{C=O}} = \frac{k_{2,m}}{k_{2,b}} \cdot \frac{K_{AH}K_B}{(\sqrt{K_{AH}}+\sqrt{K_B})^2 V} \cdot \frac{K_A^-}{K_{AH}} \qquad (12)$$

(a) (b) (c)

Factor (a) in the right-hand part of Eq (12) reflects the change in the reactivity of the substance on their being transferred from water to a micellar medium and it is equal to approx 10, as follows from Table 1. Factor (b) reflects the contribution that increasing the concentration of the reagents in the micelles makes into the acceleration of the reaction. The value of this contribution, as seen in Table 3, is 10^2, if one assumes[17] that $V = 0{\cdot}37\ M^{-1}$. And factor (c) is the maximal shift of $pK_{a2,app}$ (see Eq 4b), which is equal to $\sim 10^2$, as follows from the K_A^- and K_{AH} results presented in Table 3. As a result, the overall maximal acceleration of the reaction calculated with the help of Eq (12) is approx 10^5 times, a value not much different from 8×10^4 times (Table 1) afforded by the "k_{app}-[CTAB]" profile at pH 8·8 (Fig 2, curve **a**). This indicates that the theory (Eqs 11 and 12) correctly predicts the experimental results.

(iii) *The intrinsic reactivity of benzimidazole anion in the micellar "phase".* The true rate-constant for the reaction of benzimidazole anion and *p*-nitrophenyl heptanoate proceeding in the micellar "phase" ($k_{2,m}$) is by approximately one order of magnitude higher than the $k_{2,b}$ value for the same reaction in water (see Table 1). This fact is clearly indicative of the effect of the micellar environment. One could think that the phenomenon revealed by us (i.e. that $k_{2,m} \gg k_{2,b}$) is due to the fact that the dielectric permeability in the surface layer of cationic micelles is much lower than in water.[26] However, on the other hand, this can not be accounted for by the mechanism commonly used for such cases,[23] according to which the transient state (anion) is stabilized by an electrostatic interaction with a positive surface-charge. This is because "neutralization" of the electrostatic potential of the micelle under the action of added salt (0·12 M KNO_3) does not change in any way the true $k_{2,m}$ value (compare the values of $k_{2,m}/V$ in Table 1). Another mechanism seems to be more plausible, i.e. when benzimidazole anion is sorbed on a micelle, its nucleophilicity increases due to the weak solvation ability of the micellar medium. In this connexion, we may say that the content of water decreases from the surface layer to the hydrophobic nucleus of the micelle.[27] This concept agrees with the fact

that the rate of nucleophilic substitution S_N2 involving anions sharply increases when the reaction takes place in an organic solvent instead of water (in the case of an aprotonic solvent, by several orders).[25,28]

(iv) *Specificity towards ester.* In the light of the data by Gitler and Ochoa-Solano[8] (see also[1]), one might think it strange that the CTAB-induced micellar effect changes insignificantly in the series of *p*-nitrophenyl carboxylates studies (Fig 3), in spite of their having different binding abilities (Table 3). It should be noted thereby that the maximal acceleration of the benzimidazole anion reaction (Eq 12) weakly depends on the binding constant (K_B) of the ester, as $K_B \gg K_{AH}$ (which holds for butyrate and heptanoate); hence factor (b) is practically equal to K_{AH}/V. This means that the contribution to acceleration by increasing the reagents concentration in the micelles is mainly determined by the binding constant of the nucleophile (K_{AH}).

(v) *Mechanism of salt effect.* The inhibiting effect of added salts on ionic reaction catalysed by CTAB micelles has been described by many authors.[20-23,29] This phenomenon is explained by the fact that an increase in the counterions concentration leads to a decrease in the sorption ability of cationic micelles towards an anionic reagent.[20,22,30] This mechanism of inhibition was supported by experimental evidence of this work (see below).

Let us analyse the overall kinetic data ("k_{app}-C" profiles, Fig 5) in terms of Eq (10). To do this, it should be taken into account that there is hardly any CTAB-induced apparent pK_{a2} shift if the concentration of the added salt (KNO_3) is high.[16] Hence, Eq (10) by combining it with (3) takes on the following form:

$$k_{app} = \frac{(k_{2,m}/V)K_A\text{-}K_BC}{(1+K_A\text{-}C)(1+K_BC)} \cdot \frac{K_{a2,b}}{[H^+]} \qquad (13)$$

assuming that, like in Eq (11), the reaction rate in water may be neglected, as the acceleration of the reaction under the action of CTAB micelles is sufficiently high even if salts are added.

We have analysed in detail the "k_{app}-C" profile in the presence of 0·12 M KNO_3 in terms of Eq (13) (Fig 5, curve c); thereby the approach was the same as used in the case of Eq (8) (see Approach I in Appendix). As a result we have found, firstly, that addition of salt does not affect the true reaction rate constant in micelles (compare the values of $k_{2,m}/V$ in Table 1). Secondly, added salt does not affect either the constant of binding between CTAB micelles and *p*-nitrophenyl heptanoate (K_B, Table 3).* The only reason for the inhibiting action is that in the presence of 0·12 M KNO_3 the benzimidazole anion binds with the micelles not more effectively than electroneutral form AH (i.e. $K_{A^-} \approx K_{AH}$) (see Table 3).

*It was previously shown with the use of a different method that 0·12 M KNO_3 does not affect the binding of either electroneutral form of benzimidazole or *p*-nitrophenyl heptanoate with CTAB micelles.[16]

†See[34] and Refs cited therein.

CONCLUSION

Special attention should be paid to comparison of *true* reactivity of benzimidazole and its anion in a micellar medium. In water benzimidazole anion (A^-) has only a 10^3 times higher reactivity than the electroneutral form (AH), such as $k_{2,b} \approx 10^3\, k_{1,b}$, see Tables 1 and 2; this, by the way, agrees with the data on imidazole.[18] In a micellar medium the difference in their reactivity is over 10^6 times, $k_{2,m} \approx 10^6\, k_{1,m}$ (see Tables 1 and 2). This is due to the fact that the true values of the second order rate-constant decreases in the micelles by two orders of magnitude as compared to that in the aqueous phase in the case of electroneutral benzimidazole (Table 2), whereas with the anion the effect of the micellar medium is favourable (Table 1). The mechanism of this phenomenon was analysed in the Discussion, but we would like to point out that these facts are important for understanding certain polymer and protein effects in the imidazole action.

In the light of this paper, one may understand why many attempts to create enzyme-like catalysts on the basis of synthetic polymers having an electroneutral imidazole group were far from successful (only 30–40 times acceleration or even much less), see review[31] and some more recent works.[32,33] In such systems there is a certain active centre in which the catalytically active imidazole group is inside (or in the vicinity) of the hydrophobic cavity. It follows from the kinetic analysis that such a structure of the "enzyme model" can hardly lead to effective catalysis. In this case the positive effect of increasing the concentration of the reagents (due to the hydrophobic interaction between the substrate and the polymer catalyst) is almost entirely compensated by the usually unfavourable effect of the active centre environment, i.e. low dielectric permeability or weak solvation ability.

For the same reason, in the active centre of α-chymotrypsin, the reactivity of the imidazole group of His-57 is intensified at the expense of its interaction with the carboxylate of Asp-102, $COO^- \ldots HIm \rightleftharpoons COOH \ldots Im^-$.† As the active centre of this enzyme is hydrophobic, the polyfunctional interaction leading to the formation of the imidazole anion (possible in the transient state only) may enhance the catalytic effect by 10^6 times, as should be inferred from the data of the present work (if one proceeds from the reactivity of the electroneutral and anionic forms of benzimidazole in a hydrophobic micellar medium (see Tables 1 and 2), compare $k_{1,m}$ and $k_{2,m}$, respectively).

APPENDIX

The kinetic theory of micellar catalysis[2,11-14] allows the true values of rate constants of the second order reaction in the micellar "phase" and the binding constants of the reagents with micelles to be found from overall kinetic data (i.e. from "k_{app}-C" profiles). The general aspects of the problem were analysed in review.[2] Next we describe two aspects which proved feasible in this work.

Approach I. Eq (8) will be convenient to use in the following form:

$$\frac{C}{k_{app}-k_{1,b}}=\alpha+\beta\frac{k_{app}C}{k_{app}-k_{1,b}}+\gamma\frac{k_{app}C^2}{k_{app}-k_{1,b}}, \quad (14)$$

where

$$\alpha=1/(k_{1,m}/V)K_{AH}K_B,$$
$$\beta=(K_{AH}+K_B)/(k_{1,m}/V)K_{AH}K_B, \quad (15)$$
$$\gamma=1/(k_{1,m}/V).$$

The value of α can be found as the ordinate intercept of the curve plotted in coordinates of Eq (14), $C/(k_{app}-k_{1,b})$ vs $Ck_{app}/(k_{app}-k_{1,b})$. Then the results of the experiment may be presented in the coordinates of linear Eq (16):

$$\frac{1}{k_{app}}-\frac{\alpha}{C}\left(1-\frac{k_{1,b}}{k_{app}}\right)=\beta+\gamma C \quad (16)$$

which follows from (14). Evidently, β and γ may be found as the ordinate intercept and the slope of the corresponding straight line, plotted in coordinates of Eq (16). Finally, knowing α, β and γ one may, with the help of Eq (15) calculate the values of K_{AH}, K_B and $k_{1,m}/V$ sought for.

In certain cases when micelles cause a marked acceleration of the reaction and, hence, $k_{app} \gg k_{1,b}$, Eqs (14) and (16) may be simplified:

$$\frac{C}{k_{app}}=\alpha+\beta C+\gamma C^2 \quad (17)$$

and

$$\frac{C/k_{app}-\alpha}{C}=\beta+\gamma C. \quad (18)$$

By way of examples in Fig 7 the data on the effect of CTAB micelles on acylation of benzimidazole by *p*-nitrophenyl heptanoate are analyzed in terms of Eqs (17) and (18).

Approach II. Eq (11) is convenient to present as:

$$\mathcal{H}=(k_{2,m}/V)\cdot K_{A^-}, \quad (19)$$

where

$$\mathcal{H}=\frac{k_{app}(1+K_{AH}C)(1+K_B)}{K_BC}\cdot\frac{[H^+]}{K_{a2,b}}. \quad (20)$$

The value of $\mathcal{H}$ was calculated with the overall values of k_{app} measured experimentally ("k_{app}-C" profiles in Figs 2 and 3) and also the data on K_{AH}, K_B given in Table 3 and $pK_{a2,b}=12{\cdot}35$ (see[16]). The values of K_{A^-} have been taken from Ref 16.

By way of example, Fig 8 shows the experimental results in terms of Eq (19).

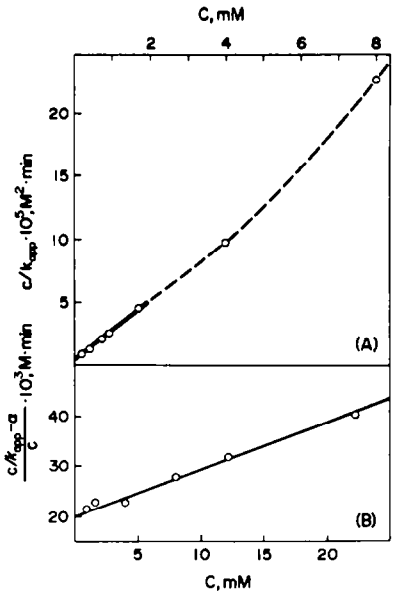


Fig 7. A graphical analysis of the "k_{app}-C" profile. Experimental results (Fig 5, curve c) are presented (A) in terms of Eq (17) and (B) in terms of Eq (18).

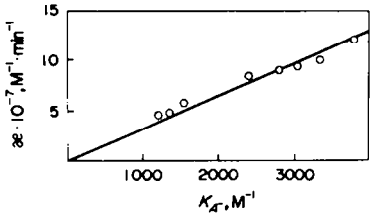


Fig 8. A graphical analysis of the "k_{app}-C" profile. Experimental results (Fig 2, curve a) are presented in terms of Eq (19).

EXPERIMENTAL

Materials. A commercial CTAB was the product of Chemapol purified by recrystallization.[35] CMC (which was equal to 6×10^{-4} M without the reagents at 25°) was determined by the electroconductivity method.[36]

SDS (Koch-Light) was purified as in ref 35. CMC determined as described[23] by the dye method with Rodamine 6 G, is equal to 5×10^{-3} M (without the reagents, 25°, 1 vol % of dimethylsulphoxide).

p-Nitrophenyl carboxylates were synthesised by Dorovska.[37] Benzimidazole, chemical grade, the product of Soyuzkhimreactiv was twice recrystallised from alcohol. N-methylbenzimidazole was synthesised as described[38] and purified by repeated distillation in vacuum. The components for the buffer solution and the analytical grade salts (Soyuzkhimreactiv) were used without purification.

Kinetics. The kinetics of the reactions studied (Schemes 1–3) were assayed spectrophotometrically, liberation of either *p*-nitrophenolate-ion (400 nm) or non-dissociated form of *p*-nitrophenol (320 nm) was followed with the help of a "Hitachi-Perkin-Elmer-124" recording spectrophotometer. The reaction was run at 30° either in 0·02 M phosphate buffer at pH < 7·5 or in 0·02 M borate buffer at pH > 7·5. A usual procedure was the

following: to a 1 cm optical cuvette containing 2·97 ml of the buffer with a certain concentration of the surfactant and benzimidazole (or N-methylbenzimidazole), 0·03 ml of the ester soln in dimethylsulfoxide were added; the initial concentration of the ester in the cuvette was from 5×10^{-6} to 4×10^{-5} M. The initial concentration of benzimidazole (from 8×10^{-5} to 5×10^{-2} M) greatly exceeded that of the ester in all experiments. Owing to such concentration ratios of the reagent, the "product vs time" curve could be analysed in terms of the pseudofirst order kinetics, $k_{obs} = k_{spont} + k_{app}$ [benzimidazole], using the method of Huggenheim. The value of k_{spont}, which corresponds to the spontaneous (alkaline) hydrolysis of the ester, did not exceed 10% of k_{obs} and was, as a rule, much lower. The second order rate constant, k_{app}, was determined from the slope of a straight line obtained if k_{obs} is plotted against the initial concentration of benzimidazole (or its N-Me derivative). The dependence of k_{app} upon the surfactant concentration was analysed in terms of the kinetic theory of micellar catalysis,[2,11-14] see also Theory and Appendix.

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