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Kinetic Solvent Deuterium Isotope Effects on the Micellar-Catalyzed Hydrolysis of Trisubstituted Phosphate Esters[1]

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Received June 25, 1975

Functional micelles of hexadecyl(2-hydroxyethyl)dimethylammonium bromide (I) are better catalysts than hexadecyltrimethylammonium bromide (CTABr) for the alkaline hydrolysis of diethyl and di-*n*-hexyl *p*-nitrophenyl phosphate (IIIa,b). The kinetic solvent deuterium isotope effects for reactions catalyzed by CTABr are very similar to those for reaction in water, but for reaction of IIIb in the presence of I the inverse isotope effect gradually disappears with increasing concentration of hydroxide ion. These results show that the inverse isotope effect is due to the ionization of I to its zwitterion II at high pH. They are consistent with nucleophilic attack by the alkoxide moiety in II but not with general acid or base catalysis.

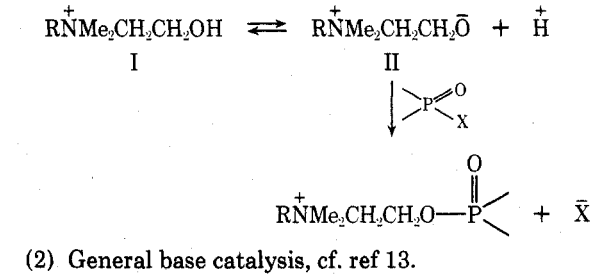
Micelles catalyze (or inhibit) many bimolecular reactions in aqueous solvents.[3] The catalysis can be explained in terms of the ability of the micelle to bring the reagents together at its surface in an environment which is favorable to reaction with stabilization of the transition state and avoidance in part of the unfavorable entropy effects caused by forming an activated complex from two or more reagents.[8] In general the catalysis is greater if one reagent is chemically incorporated into the surfactant, by analogy with the greater ease of intra- as compared with intermolecular reactions.[8] Most functional surfactants have contained amino or thiol groups and the former could act as nucleophiles or general bases,[4-6] but we have used quaternary ammonium ions derived from ethanolamine as reagents in reactions of phosphate esters[9,10] and acyl phosphates. Our evidence is consistent with the surfactant (I) generating the zwitterion (II) which reacts as a nucleophile. These micellized surfactants are effective reagents toward saturated and carbonyl carbon, and it was suggested that here they acted by increasing the nucleophilicity of hydroxide ion.[11]

Micelles of I can be regarded as models of protein bond serine, whose nucleophilicity is important in many enzymic reactions,[12] so that it is important to distinguish between the possible modes of catalysis in reactions catalyzed by micelles of I and related surfactants.

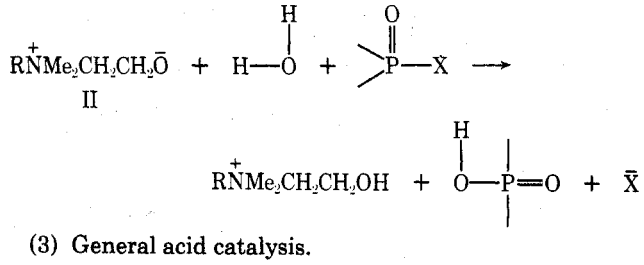
There are four reasonable mechanisms (1–4) by which micelles of I could speed reactions. They are shown for reaction at a phosphoryl group, but similar paths can be written for some of the reactions at carbon.

The fact that micelles of I are no better catalysts than micelles of cetyltrimethylammonium bromide (CTABr) for attack of fluoride ion upon *p*-nitrophenyl diphenyl phosphate[10] argues against 3 and 4, but either 1 or 2 are consistent with the evidence.

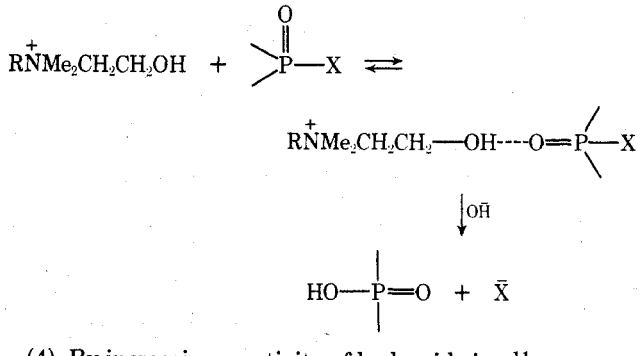
(1) Nucleophilic attack.[9,10]



(2) General base catalysis, cf. ref 13.



(3) General acid catalysis.



(4) By increasing reactivity of hydroxide ion.[11]

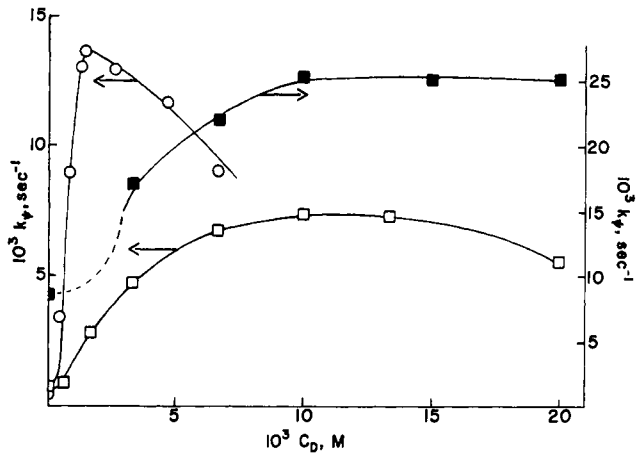
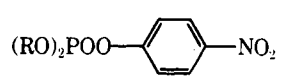


Figure 1. Reactions of *p*-nitrophenyl phosphate esters in 0.01 *M* NaOH at 25.0°. The open symbols are for reactions in CTABr in 0.01 *M* NaOH and the closed for reactions in the hydroxyethyl surfactant I in 0.1 *M* NaOH. Reactions of ■, □, diethyl (IIIa); ○, di-*n*-hexyl (IIIb).

Kinetic deuterium solvent isotope effects should distinguish between these mechanisms,[14] because those involving general catalysis should show relatively large effects with $k_{H_2O}/k_{D_2O} > 2$.[13,16,17] If the micelle merely increased the reactivity of hydroxide ion the solvent isotope effect should be typical of nucleophilic attack by that ion, but mechanism 1 predicts that the isotope effect will vary with hydroxide ion concentration, because of the changing equilibrium between I and II, although the overall isotope effect should not be large.[18]

In earlier work we used *p*-nitrophenyl diphenyl phosphate as substrate and followed the reaction using a stopped flow spectrometer,[9,10] but here we used the less reactive diethyl and di-*n*-hexyl *p*-nitrophenyl phosphate (IIIa,b) so that the rates in D_2O could be measured conventionally.



III

(IIIa, b, R = Et, *n*-Hex, respectively)

Experimental Section

Materials. The preparation and purification of the substrates and surfactant followed standard methods.[9,19] Redistilled and deionized water was used in the rate measurements.

Kinetics. The slower reactions were followed spectrophotometrically at 25.0° using Gilford spectrophotometers with water jacketed cell compartments,[9,10] and the faster reactions were followed in a Durrum stopped flow spectrophotometer with a Biomation 805 data acquisition unit. The substrate concentrations were ca. 10^{-5} *M*.

The kinetics were first order and the observed first-order rate constants, k_ψ, are in sec^{-1}, at 25.0°. In the figures we denote the surfactant (detergent) concentration as C_D.

Results

Micellar effects upon the reactions of *p*-nitrophenyl diphenyl phosphate but not of IIIa,b have been examined.[9,10,20]

It was difficult to follow the reaction of *p*-nitrophenyl di-*n*-hexyl phosphate (IIIb) in water in the absence of surfactant because of its low solubility, and using 1.8×10^{-6} *M* substrate in 0.1 *M* NaOH the initial part of the first-order plot was curved, but from the final linear part we calculated $k_\psi \sim 7.5 \times 10^{-4}$ sec^{-1}; with 5 vol % dioxane there was less bending and the linear part of the curve gave $k_\psi \sim 7.7 \times 10^{-4}$ sec^{-1} and in 12 vol % dioxane a good first-order plot

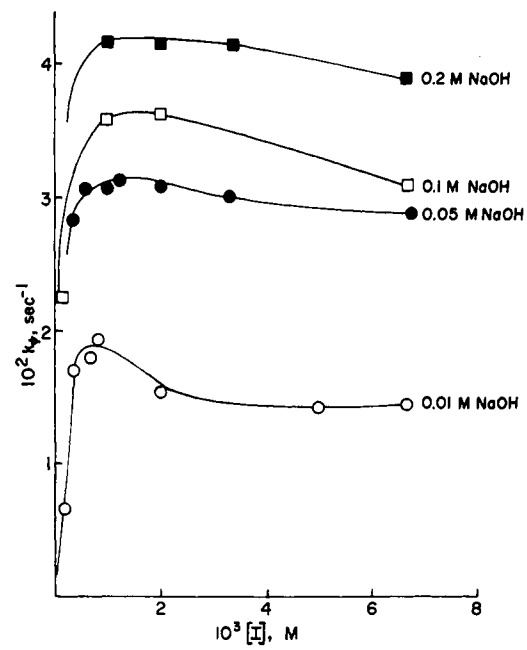


Figure 2. Effect of hydroxide ion on reaction of *p*-nitrophenyl di-*n*-hexyl phosphate (IIIb) in the presence of the hydroxyethyl surfactant I.

was obtained with $k_\psi = 7.7 \times 10^{-4}$ sec^{-1}. The initial curvature was probably due to the low solubility of the ester, so that first-order kinetics were not observed until all the ester was in solution, and it and the diethyl compound have similar reactivities in the absence of surfactant.

Relatively small amounts of dioxane have little effect on the reaction rate, and for reaction of *p*-nitrophenyl diethyl phosphate in 0.01 *M* NaOH at 25.0°, $10^5 k_\psi$ = 8.5, 8.7, 8.8, and 8.6 sec^{-1} in water and 5, 8, and 12% dioxane by volume, respectively. Addition of organic solvents can assist bimolecular nucleophilic attack because of decreased hydration of the nucleophile.[21] Probably the absence of rate enhancement here is related to a decrease in the activity coefficients of the hydrophobic substrates which offsets the normal solvent effect, and dioxane retards reaction of IIIb.

Micellar Effects. Cationic micelles of the nonfunctional surfactant, CTABr, catalyze reactions of IIIa,b and the rate constant–surfactant profile (Figures 1 and 2 and Table I) is similar to that for the hydrolysis of *p*-nitrophenyl diphenyl phosphate.[9,10] The less hydrophobic ester bonds less to micelles and more surfactant is needed to reach rate maxima in reactions of the diethyl as compared with the di-*n*-hexyl phosphate.[4-7] The maximum rates in micelles of CTABr are different for the two esters, with the more hydrophobic dihexyl compound being the more reactive (Table I). In many micellar-catalyzed reactions both reagent–micelle binding and the rates in the micelle increase with increas-

Table I
Micellar Effects upon Reactions of Phosphate Triesters[a]

Substrate	Surfactant				
	CTABr	I			
[OH$^-$], *M*	0.01	0.01	0.05	0.1	0.2
Diethyl	9			3	
Di-*n*-hexyl	18	256	84	49	28
Diphenyl[b]	12	310	104	58	

[a] Values of the rate enhancement at 25.0° and surfactant concentration at the rate maximum. In the absence of surfactant the second-order rate constants are respectively diethyl, 8.5×10^{-3}; di-*n*-hexyl, 7.5×10^{-3}; diphenyl, 0.49 l. mol^{-1} sec^{-1}. [b] Reference 20.

Table II
Deuterium Solvent Isotope Effects in Reactions of Phosphate Esters [a]

Substrate	Surfactant	$[\bar{O}H]$, M [b]	$10^3 k_{H_2O}$	$10^3 k_{D_2O}$	k_{H_2O}/k_{D_2O}
Diethyl		0.01	0.0853	0.0982	0.87
Diethyl	10^{-2} M CTABr	0.10	5.43	5.78	0.94
Diphenyl		0.01	4.86	4.43	1.10
Diphenyl	1.5 mM CTABr	0.01	96.2	75.7	1.27
Diphenyl	1.5 mM CTABr	0.05	263	231	1.14
Di-*n*-hexyl	1.5 mM CTABr	0.01	3.02	3.23	0.93
Di-*n*-hexyl	2 mM I	0.01	15.4	20.4	0.75
Di-*n*-hexyl	2 mM I	0.10	36.0	40.6	0.89
Di-*n*-hexyl	2 mM I	0.15	39.1	41.0	0.95
Di-*n*-hexyl	2 mM I	0.20	41.6	41.6	1.00

[a] At 25.0°; $[O\bar{D}]$ in D_2O.

ing hydrophobicity of the reagent, which would be expected if drawing the reagents deeper into the Stern layer of the micelle increases beneficial Coulombic interactions with the charged head groups.[4-7,22] The dependence of micellar catalysis with substrate hydrophobicity is often much larger than that seen here, suggesting that reaction occurs in a water-rich region at the micellar surface, with interactions between water and the anionic transition state, which is consistent with the observation that nonionic micelles strongly inhibit reactions of *p*-nitrophenyl diphenyl phosphate with hydroxide or fluoride ion.[20]

The hydroxyethyl surfactant (I) is a considerably better catalyst than CTABr, in agreement with earlier evidence, and the variation of the maximum values of k_ψ with hydroxide ion concentration is similar to that observed earlier and can be explained in terms of an equilibrium between I and II.[10]

At similar pH micelles of the hydroxyethyl surfactant (I) which are converted partially into the zwitterion II are considerably better reagents than hydroxide ion in CTABr, and functional micelles are generally better catalysts than nonfunctional micelles, because of the more favorable entropies of activation. However, in the absence of micelles the cholinate ion is also a better nucleophile than hydroxide ion,[10] suggesting that the quaternary ammonium centers in micellar and nonmicellar systems interact favorably with the anionic moieties in the transition state (cf. ref 23).

Kinetic Deuterium Solvent Isotope Effects. Because of differences in D_2O and H_2O as solvents,[15b] there could be an isotope effect on the properties of the micelles and their interactions with solutes. However, where kinetic solvent isotope effects have been measured in the presence of micelles, for example in acid-catalyzed reactions,[24] the isotope effects have been similar to those expected by analogy with measurements in the absence of micelles. The kinetic solvent isotope effects upon reactions of diethyl and *p*-nitrophenyl diphenyl phosphate in water and CTABr are similar (Table II), suggesting that here too the isotope effect depends on mechanism rather than on micellar properties.

In many reactions of lyate ion, there is a small inverse deuterium isotope effect,[18] because OD^- is a better nucleophile than OH^- as well as being a stronger base. Our results with *p*-nitrophenyl diethyl phosphate accord with these observations, but there is a small normal isotope effect on the reaction of *p*-nitrophenyl diphenyl phosphate, probably owing to differences in solvation, although the fastest of these reactions was only just within the range of measurement by conventional methods.

The mechanistically distinctive observations are on the reactions of *p*-nitrophenyl di-*n*-hexyl phosphate (IIIb). In CTABr the isotope effect is the same for this reaction and that of the diethyl compound (Table II). (We did not attempt to measure k_{H_2O}/k_{D_2O} for reaction of IIIb in water because of its low solubility.) In the presence of micellized hydroxyethyl surfactant I the inverse isotope effect gradually disappears with increasing concentration of lyate ion (Table II).

The equilibrium between I and the zwitterion II should move in favor of the latter with a change of solvent from protium to deuterium oxide, leading to the inverse deuterium solvent isotope effect. With increasing stoichiometric concentration of lyate ion this conversion should increase and as it becomes complete the observed kinetic solvent isotope effect will not depend upon the acid–base equilibrium between I and II, but only upon the kinetic isotope effects on the reaction of the alkoxide moiety in II with the substrate and any effects caused by differences in properties of the micelles in H_2O and D_2O, and to a first approximation these effects should not change with the stoichiometric concentration of lyate ion.

Therefore for nucleophilic catalysis the solvent deuterium isotope effect k_{H_2O}/k_{D_2O} should be inverse and close to unity, and increase to a constant value with increasing hydroxide ion concentration.

This change in the kinetic solvent isotope effect is fully consistent with mechanism 1 and it does not fit mechanisms involving general acid or base catalysis. For example, in reactions of *p*-nitrophenyl diphenyl phosphate catalyzed by functional micelles derived from histidine[13] or imidazole,[25] we observe $k_{H_2O}/k_{D_2O} \sim 2.5$, which is consistent with the imidazole moiety acting as a general base, and small isotope effects are observed when histidine or imidazole acts as a nucleophile.

We have no model on which to predict the solvent isotope for mechanism 4 although it is difficult to see how it could explain the systematic change in the isotope effect with increasing hydroxide ion concentration.

Registry No.—I, 20317-32-2; IIIa, 311-45-5; IIIb, 57016-65-6; *p*-nitrophenyl diphenyl phosphate, 10359-36-1.

References and Notes

(1) Support of this work by the Arthritis and Metabolic Diseases Institute of the U.S. Public Health Service is gratefully acknowledged.
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Micellar Effects on the Hydrolysis of *p*-Nitrobenzoyl Choline and the Related *N*-Hexadecyl Ester[1]

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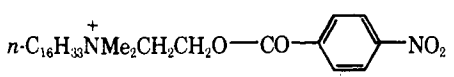
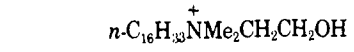
Received June 25, 1975

The reaction of hexadecyl(2-hydroxyethyl)dimethylammonium bromide *p*-nitrobenzoyl ester (n-$C_{16}H_{33}N^+Me_2CH_2CH_2OCOC_6H_4NO_2Br^-$, I) with hydroxide ion is catalyzed to similar extents by cationic micelles of hexadecyltrimethylammonium bromide (CTABr) or hexadecyl(2-hydroxyethyl)dimethylammonium bromide (n-$C_{16}H_{33}NMe_2CH_2CH_2OHBr^-$, II), suggesting that the greater catalytic efficiency of II in other reactions is due to nucleophilic attack by the zwitterion generated by II at high pH. Saponification of the more hydrophilic *p*-nitrobenzoyl choline (III) is not micellarly catalyzed. In the absence of surfactant I is slightly more reactive than III in aqueous dioxane and the reactions slow as the solvent becomes more aqueous, but there is a rate minimum in reactions of I at ca. 95 mol % water, and the rate increases in more aqueous solvents owing to association of I.

The biological importance of acetyl choline makes choline esters interesting substrates for mechanistic work[2] and a systematic study has been made of substituent effects upon reactions of hydroxide ion with benzoyl cholines.[3] We have kinetic evidence for the intermediacy of a related ester, hexadecyl(2-hydroxyethyl)dimethylammonium bromide *p*-nitrobenzoyl ester (I), in reactions of *p*-nitrobenzoyl phosphate in dilute alkali catalyzed by micelles of the choline derivative (II),[4] and we were therefore interested in the hydrolysis of I.



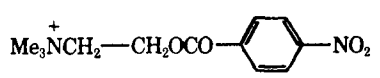
I



II

Micelles of II are effective catalysts of phosphate ester hydrolysis,[9] and of SN2 and E2 reactions at saturated carbon, and of reactions of carboxylic esters and amides.[10]

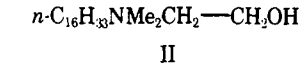
We also compared the reactivity of I with that of *p*-nitrobenzoyl choline (III), because self-micellization of I could make it more reactive than III toward anionic nucleophiles.



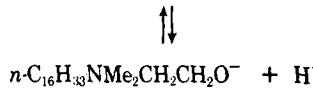
III

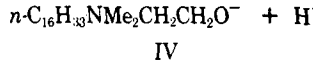
There has been considerable work on the reactions of esters of long-chain n-alkane carboxylic acids with n-alkyl amines and related compounds.[11-14] Comicellization has been shown to be of great importance in many of these reactions, and both the overall reaction rate and the kinetic form changes when the reactants are micellized.[11,12] Substrate micellization is also of great importance in hydrolysis of monoalkyl sulfates in both acidic and basic media.[15] Many biological reactions occur at interfaces, and the use of chemically inert micelles and micellized reactants can give evidence on the role of these microscopic medium effects;[5-8,16] for example, the hydroxyethyl head group of II can be regarded as a model for the corresponding group in serine which is implicated as a nucleophile in many enzymic reactions.[17]

In this work we compare reactivities of I and III in the presence of micelles of the cationic surfactants (detergents), hexadecyltrimethylammonium bromide (CTABr) and II. At high pH micelles of the choline-derived surfactant II are effective reagents for the decomposition of di- and trisubstituted phosphate esters, and it was suggested that the alkoxide moiety of the zwitterion IV attacked the phosphoryl group.[9b]



II





IV

Although in nucleophilic reactions involving II[10,18] the alkoxide moiety could act as a nucleophile, other reasonable mechanisms can be postulated.[9,10] For example, the hydroxyl moiety could act as a general acid and assist attack of hydroxide ion, or the alkoxide moiety in IV could act as a general base and activate a water molecule (cf. ref 19), and it has also been suggested that micelles of II activate hydroxide ions.[10] The kinetic form of the reaction and its pH–rate profile do not distinguish between these possibilities.

Nucleophilic attack by IV upon I gives no chemical change, and in that event micelles of II should be no better catalysts than micellized CTABr. However, if II is more ef-