Secondary Valence Force Catalysis. II. Kinetics of the Hydrolysis of Orthoesters and the Hydrolysis and Aminolysis of Carboxylic Esters in the Presence of Micelle-Forming Detergents[1]

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The hydrolysis of methyl orthobenzoate is subject to marked catalysis by dilute aqueous solutions of sodium lauryl sulfate and other anionic detergents. Below the critical micelle concentration for sodium lauryl sulfate, the rate of the catalyzed reaction increases with approximately the fourth power of detergent concentration, suggesting the formation of substrate-induced micelles. The hydrolysis of ethyl orthovalerate and ethyl orthopropionate, but not that of ethyl orthoformate, is subject to modest catalysis by sodium lauryl sulfate. The reactions of p-nitrophenyl hexanoate and, to a lesser extent, those of p-nitrophenyl acetate with hydroxide ion and leucine are catalyzed by cetyltrimethylammonium bromide. The reactions of p-nitrophenyl hexanoate with leucine and hydroxide ion are inhibited by sodium lauryl sulfate and a nonionic detergent and the reaction of this substrate with morpholine is inhibited by cetyltrimethylammonium bromide as well as by the above detergents. The rates for the corresponding reactions with p-nitrophenyl acetate are relatively insensitive to the presence of these detergents.

Introduction

The theoretical and practical reasons for the study of kinetics of organic reactions in micelles have been presented by Duynstee and Grunwald.[2] The relationship of such studies to enzyme-catalyzed reactions has been indicated in the preceding communication.[3] In this communication, the results of a study of the kinetics of the hydrolysis of orthoesters and of the hydrolysis and aminolysis of carboxylic esters in the presence of micelle-forming detergents are recorded.

Experimental

Materials. Methyl orthobenzoate was prepared from benzotrichloride (α,α,α-trichlorotoluene) as previously described.[4] Ethyl orthoformate and ethyl orthopropionate were obtained commercially and redistilled before use. Ethyl orthovalerate was prepared from valeronitrile by a standard procedure.[5] *p*-Nitrophenyl acetate and *p*-nitrophenyl hexanoate were prepared by the method described by Bender and Nakamura.[6] Other organic reagents were recrystallized or redistilled before use. Sodium lauryl sulfate and cetyltrimethylammonium bromide were commercial products and were purified before use.[2] The nonionic detergent (NID) composed of dodecylphenol condensed with 18 molecules of ethylene oxide was a gift of the General Aniline and Film Corp. and was employed without further purification. Sodium oleyl sulfate and sodium heptadecyl sulfate were obtained from commercial sources and were used without further purification. Deuterium oxide was redistilled before use.

Kinetic measurements for the hydrolysis of methyl orthobenzoate and for the ester hydrolysis and aminolysis reactions were carried out spectrophotometrically at 25° as previously described.[7,8] The hydrolysis of the remaining orthoesters was followed by the hydroxylamine–ferric chloride method of Lipmann and Tuttle.[9] Values of pH were determined with a glass electrode and a Radiometer PHM 4c pH meter. Values of pD were obtained from measured values of pH and the relationship: pD = pH + 0.40.[10] This relationship was verified for our pH meter using carefully prepared acetate buffers.

Results

In Figure 1, first-order rate constants for the hydrolysis of methyl orthobenzoate in aqueous solution at 25° are plotted as a function of the concentration of sodium lauryl sulfate (NaLS), sodium oleyl sulfate (NaOS), sodium heptadecyl sulfate (NaHS), and cetyltrimethylammonium bromide (CTAB). All reactions were carried out at pH 4.76. The first-order rate constants increase very rapidly with increasing NaLS and NaOS concentration and slightly with increasing NaHS concentration. In contrast, 0.04 *M* CTAB inhibits this reaction about threefold. The rate of methyl orthobenzoate hydrolysis is unaffected by 0.02 *M* sodium sulfate and 0.036 *M* sodium methyl sulfate. The catalysis of this reaction by NaLS was chosen for more thorough investigation.

The results of an extended study of the variation in first-order rate constant for methyl orthobenzoate hydrolysis as a function of NaLS concentration are collected in Table I. Below the critical micelle concentration for this detergent, approximately 0.0016 *M*,[11] the first-order rate constants for the catalyzed reaction increase considerably more rapidly than the

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Table I. Effect of Sodium Lauryl Sulfate Conce tra ion on the First-Order Rate Constants for the Hydrolysis of Several Orthoesters at 25°

	k_{obsd}, min.$^{-1}$					
	Methyl orthobenzoate[a]			Ethyl ortho-formate[b]	Ethyl ortho-propionate[c]	Ethyl ortho-valerate[d]
NaLS, *M*	pH 4.76	pH 5.10	pH 5.65	pH 6.63	pH6.68	pH 6.65
0.00	0.064	0.044	0.0087	0.11	0.22	0.24
0.0004	0.083					
0.0008	0.13					
0.0012	0.47					
0.0016	1.2					
0.0020	1.3					
0.0040	3.8		0.15			
0.0060	4.9					
0.0090						0.33
0.010	5.3	1.51	0.44			
0.020			0.72			
0.0225				0.091		
0.030			0.28			
0.045				0.080	0.51	1.1
0.060		0.96				
0.080		0.92				

[a] Substrate concentration 0.0005 *M*. [b] Substrate concentration 0.06 *M*, run in 25% acetonitrile. [c] Substrate concentration 0.013 *M*, run in 25% acetonitrile. [d] Substrate concentration 0.010 *M*, run in 25% acetonitrile.

Table II. Fir t-Order Rate Constants for the Hydrolysis of Methyl Orthobenzoate in Water and Deuterium Oxide in the Presence of 0.01 *M* NaLS as a Function of pH and pD[a]

pH	k_{obsd}, min.$^{-1}$	pD	k_{obsd}, min.$^{-1}$
4.73	3.20	4.50	8.3
5.31	0.92	5.18	3.5
6.17	0.15	5.77	0.49
6.46	0.058	6.09	0.38
6.83	0.023	6.72	0.065

[a] $k_H = 1.7 \times 10^5\ M^{-1}$ min.$^{-1}$ $k_D = 3.5 \times 10^5\ M^{-1}$ min.$^{-1}$

first power of detergent concentration. A plot of the observed rate constants, less the value obtained in the absence of detergent, against the fourth power of the detergent concentration yields an approximately straight line. The estimate of the catalyzed rate obtained by subtracting the rate constant in the absence of detergent from those in the presence of detergent is not strictly legitimate since the contribution of the uncatalyzed reaction will vary as a function of detergent concentration. However, at low detergent concentrations this approximation will not introduce a large error since, probably, only a small fraction of the orthoester is complexed with the detergent. At high detergent concentrations, the magnitude of the correction is very small compared with the observed rate constants and, hence, no appreciable error is introduced in applying this procedure.

Above the critical micelle concentration, the first-order rate constants for methyl orthobenzoate hydrolysis continue to increase, although considerably more slowly, and, as indicated by the data at pH 5.10 and 5.65, eventually level off and finally decrease with increasing detergent concentration. The maximum catalysis observed for this reaction amounts to an 85-fold increase over the rate in the absence of detergent.

First-order rate constants for the hydrolysis of methyl orthobenzoate at pH 4.95 and 25° in the presence of 0.001 *M* NaLS are presented in Figure 2 as a function of substrate concentration. At very low substrate concentrations, the first-order rate constants are independent of this variable but decrease thereafter with increasing substrate concentration and eventually

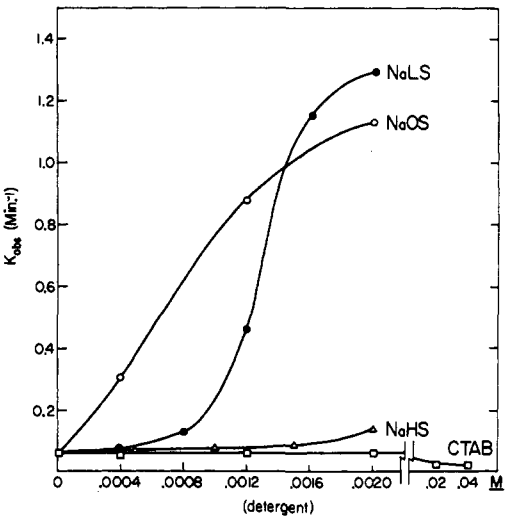

Figure 1. First-order rate constants for the hydrolysis of methyl orthobenzoate in aqueous solution at 25° and pH 4.76 plotted as a function of the concentration of sodium lauryl sulfate (●), sodium oleyl sulfate (○), sodium heptadecyl sulfate (△), and cetyltrimethylammonium bromide (□).

approach the value for this reaction in the absence of detergent (dotted line in Figure 2).

First-order rate constants for the hydrolysis of methyl orthobenzoate in the presence of 0.01 *M* NaLS at various values of pH and pD are collected in Table II. The first-order rate constants for the NaLS-catalyzed hydrolysis increase linearly with hydrogen ion concentration as do those for the reaction in the absence of detergent.[12-14] A solvent deuterium isotope effect

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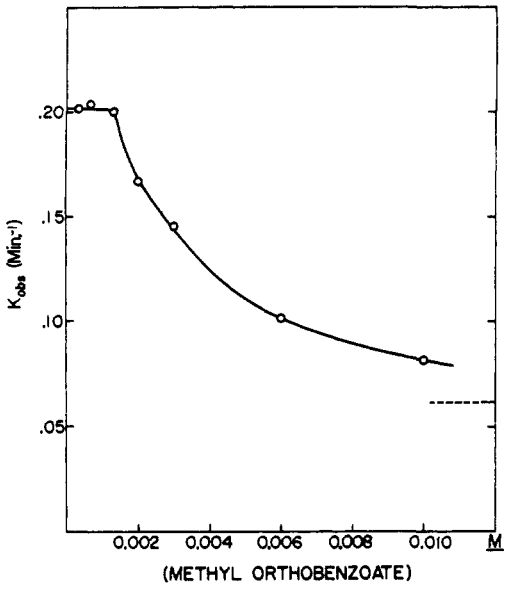

Figure 2. First-order rate constants for the hydrolysis of methyl orthobenzoate in the presence of 0.001 *M* sodium lauryl sulfate at 25° and pH 4.95 plotted as a function of substrate concentration. The dotted line indicates the rate constant under these conditions in the absence of detergent.

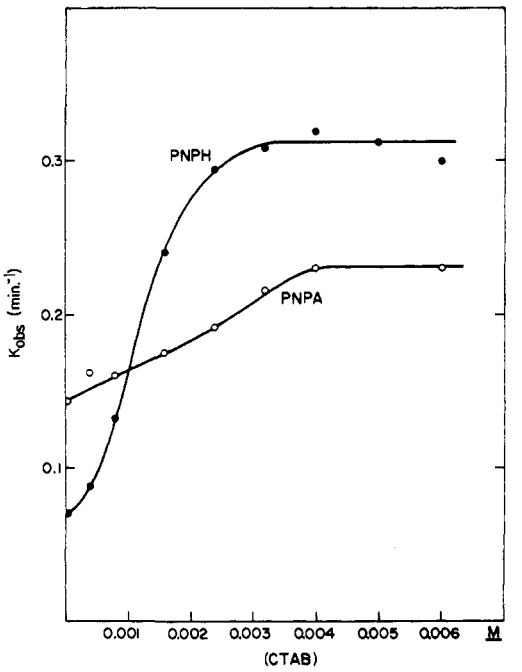

Figure 3. First-order rate constants for the hydrolysis of *p*-nitrophenyl acetate (○) and *p*-nitrophenyl hexanoate (●) at 25° at pH 10.07 plotted as a function of the concentration of cetyltrimethylammonium bromide.

for the catalyzed reaction, k_H/k_D = 0.48, was calculated from the data in this table. This value is similar to that previously obtained for this reaction in the absence of detergent.[13,14]

First-order rate constants for the hydrolysis of ethyl orthoformate, ethyl orthopropionate, and ethyl orthovalerate in the absence and in the presence of varying concentrations of NaLS are also collected in Table I. Under the conditions of these experiments, the hydrolysis of ethyl orthovalerate and ethyl orthopropionate, but not that of ethyl orthoformate, is subject to modest catalysis in the presence of NaLS. The catalysis is more marked in the case of ethyl orthovalerate as substrate. These data were obtained employing much higher substrate concentrations than in the case of the methyl orthobenzoate studies.

First-order rate constants for the base-catalyzed hydrolysis of *p*-nitrophenyl acetate (PNPA) and *p*-nitrophenyl hexanoate (PNPH) at 25° and pH 10.07 are plotted against the concentration of CTAB in Figure 3 (see also Table III). First-order rate constants for the detergent-catalyzed hydrolysis of PNPH increase more rapidly than the concentration of detergent at low detergent concentrations, and level off above the critical micelle concentration (approximately 0.001 *M*).[11] The maximum observed catalysis is approximately fivefold. In contrast, the first-order rate constants for the hydrolysis of PNPA are much less sensitive to the presence of CTAB. Under conditions in which the rate of hydrolysis of PNPH is accelerated nearly fivefold, the rate of hydrolysis of PNPA is increased only 65%. Under the same conditions, the first-order rate constants for the hydrolysis of *p*-nitrophenyl hexanoate are markedly decreased in the presence of 0.01 *M* NaLS and 0.01 *M* NID (Table III).

First-order rate constants for the appearance of *p*-nitrophenolate from *p*-nitrophenyl acetate and *p*-nitrophenyl hexanoate in the presence of 0.02 *M* leucine at pH 9.70 and in the presence of 0.10 *M* morpholine at pH 9.20 are collected in Table IV. These reactions were studied in the presence and absence of the various detergents. The first-order rate constants in Table IV have been corrected, in most cases, for the concomitant hydrolysis reaction, employing the data in Table III and the knowledge that the hydrolysis of these substrates is base catalyzed in this pH region. The rate constants for reactions of *p*-nitrophenyl acetate in the presence of NaLS and NID have not been corrected for the hydrolysis reaction but an examination of the data in Tables III and IV strongly suggests that these corrections should be quite small, particularly in the reactions involving morpholine. The attack of leucine on *p*-nitrophenyl hexanoate is accelerated more than tenfold in the pres-

Table III. The Effect of Concentration of Several Detergents on the Rate of Hydrolysis of *p*-Nitrophenyl Acetate and *p*-Nitrophenyl Hexanoate in Aqueous Solution at 25° and pH 10.07

Detergent, *M*	k_{obsd}, min.$^{-1}$ PNPA[a]	PNPH[b]
None	0.14	0.069
CTAB, 0.0004	0.16	0.087
0.0008	0.16	0.13
0.0016	0.18	0.24
0.0024	0.19	0.30
0.0032	0.22	0.31
0.0040	0.23	0.32
0.0050		0.29
0.0060	0.23	
NaLS, 0.005		0.0098
0.010		0.0045
NID, 0.010		0.050
0.050		0.016

[a] *p*-Nitrophenyl acetate. [b] *p*-Nitrophenyl hexanoate.

Table IV. The Effect of Several Detergents on the Rate of Reaction of *p*-Nitrophenyl Acetate and *p*-Nitrophenyl Hexanoate with Leucine and Morpholine in Aqueous Solution at 25°

Nucleophilic reagent	k_{obsd}, min.$^{-1}$[a] PNPA	PNPH
Leucine[b]		
No detergent	0.16	0.056
0.01 *M* CTAB	0.49	0.59
0.01 *M* NaLS	0.17[d]	0.0048
0.01 *M* NID	0.17[d]	0.003
Morpholine[c]		
No detergent	1.93	0.40
0.004 *M* CTAB	1.85	0.25
0.01 *M* CTAB	1.66	0.17
0.004 *M* NaLS	1.60[d]	0.13
0.01 *M* NaLS	1.54[d]	0.089
0.004 *M* NID	1.57[d]	0.11
0.01 *M* NID	1.34[d]	0.069

[a] Corrected for contribution, less than 20% in most cases, of the hydrolysis reaction. [b] Total leucine concentration 0.02 *M*, pH 9.70. [c] Total morpholine concentration 0.1 *M*, pH 9.20. [d] Uncorrected for hydrolysis reaction.

ence of 0.01 *M* CTAB compared with a threefold rate acceleration in the case of the acetate. Similarly, the first-order rate constants for leucine attack on the hexanoate are strongly decreased in the presence of 0.01 *M* NaLS and 0.01 *M* NID while those for the acetate are relatively unaffected by the presence of these detergents.

First-order rate constants for the attack of morpholine on *p*-nitrophenyl acetate are quite insensitive to the presence of 0.01 *M* concentrations of any of the detergents. In contrast, the corresponding values for the reactions of the hexanoate are decreased, to varying extents, by each of the detergents.

Discussion

Hydrolysis of Orthoesters. Below the critical micelle concentration for NaLS, the first-order rate constants for the NaLS-catalyzed hydrolysis of methyl orthobenzoate increase with approximately the fourth power of detergent concentration. Since detergents appear to exist as monomers below the critical micelle concentration,[15] this finding suggests that methyl orthobenzoate causes the formation of "induced-micelles" containing substrate and detergent in a molar ratio of approximately 1:4. Such substrate-induced formation of a catalytically active structure may be considered a model for substrate-induced conformation changes in enzymes thought to occur in at least some enzyme-catalyzed reactions.[16] Micellation of NaLS has been demonstrated to occur below the c.m.c. for this detergent in the presence of certain charged dyes,[17] and β-naphthol causes induced micellation of CTAB.[18]

At higher concentrations of NaLS, first-order rate constants for methyl orthobenzoate hydrolysis continue to increase slowly, level off, and finally decrease somewhat with increasing NaLS concentration. Although other factors must also be important, this behavior suggests saturation of substrate with catalyst. A related dependence of rate on catalyst concentration has been observed for the chymotryptic hydrolysis of PNPA.[19] A quantitative interpretation of the dependence of first-order rate constants for methyl orthobenzoate hydrolysis upon NaLS concentration is not possible with the data at hand. Such an analysis would require knowledge of equilibrium constants for formation of "induced micelles," the equilibrium constant for the incorporation of substrate into the ordinary micelle, and the concentration of monomer in equilibrium with the micelles above the critical micelle concentration.

Further evidence for the association of substrate with detergent below the c.m.c. is provided by the variation in first-order rate constant for methyl orthobenzoate hydrolysis in the presence of 0.001 *M* NaLS as a function of substrate concentration. At very low substrate concentrations, the first-order rate constants are independent of this variable but decrease thereafter with increasing orthoester concentration and finally approach the rate constant for this reaction in the absence of detergent. This result strongly suggests saturation of the catalyst with substrate; the decreasing rate constants with increasing substrate concentration reflecting the progressively smaller fraction of substrate bound to the detergent molecules. The observed dependence of first-order rate constant on substrate concentration is qualitatively similar to the Michaelis–Menten kinetics typical of most enzymatic reactions.

The dependence of rate on hydrogen ion concentration and the magnitude of the solvent deuterium isotope effect for methyl orthobenzoate hydrolysis are nearly identical in the presence and in the absence of NaLS. These results suggest that the detergent-catalyzed reaction proceeds *via* a pathway similar to that for the uncatalyzed reaction, probably involving acid-catalyzed, rate-determining carboxonium ion formation.[13,14] In light of this probable mechanism, there are three reasonable alternatives for the observed catalysis by anionic detergents. First, the catalysis may result from electrostatic stabilization of the developing carboxonium ion by the negative charges of the micelle or "induced micelle." Second, the catalysis may result from a locally increased concentration of hydrogen ions in the immediate vicinity of the substrate–detergent complex. Finally, the catalysis may be the consequence of general acid catalysis by sodium lauryl sulfuric acid in the micellar or "induced-micellar" phases.

The hydrolysis of orthoesters derived from aliphatic acids is also subject to catalysis by NaLS, although the catalysis is less marked. Under the conditions of these experiments, the NaLS-catalysis exhibits some specificity for the substrate. The catalysis is more marked for ethyl orthovalerate than for ethyl orthopropionate and is absent in the case of ethyl orthoformate. These results appear reasonable since it is likely that the association of orthoesters with the micelle is largely the result of apolar bond formation[20] which is the primary factor leading to stability of the micelles themselves. Thus, the extent of substrate incorporation into the micellar phase is expected to increase with increasing length of a hydrophobic side chain, an expectation in

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accord with the kinetic results obtained for orthoester hydrolysis.

Hydrolysis and Aminolysis of Esters. Although other factors, indicated in the preceding communication,[3] almost certainly contribute to the observed rate effects to some extent, the alterations in the rate of ester hydrolysis and aminolysis in the presence of detergents can be qualitatively accounted for on the basis of the following reasonable assumptions. (1) *p*-Nitrophenyl hexanoate is incorporated into the micellar phases to a greater extent than *p*-nitrophenyl acetate. (2) Attractive electrostatic interactions between charges on the cationic micelle and negatively charged nucleophilic reagents cause an increased concentration of these species in the immediate vicinity of the micelle compared to the concentration of these species in the bulk phase. (3) Electrostatic repulsions and a decreased capacity for solubilization cause a decreased concentration of negatively charged nucleophilic reagents, compared to the concentration in the bulk phase, in the immediate vicinity of the anionic and nonionic micelles, respectively.

The assumption that the hexanoate is incorporated into the micellar phases to a greater extent than the acetate is rationalized on the basis of apolar bond formation as a primary driving force for the association of organic molecules with micelles and is in accord with the findings for catalysis of orthoester hydrolysis discussed above. This assumption accounts for the observation that the qualitative effects of the presence of detergents on the rate of ester hydrolysis or aminolysis is consistently more marked in the case of the hexanoate than in the case of the acetate. There is no apparent reason to suspect that, once incorporated into the micellar phases, the hexanoate and the acetate would exhibit marked quantitative differences in their behavior toward nucleophilic reagents.

Since the first-order rate constants for the CTAB-catalyzed hydrolysis of *p*-nitrophenyl hexanoate increase more rapidly than detergent concentration below the c.m.c. for this detergent, this substrate, like methyl orthobenzoate, appears to induce micellation.

The second and third assumptions account for the fact that reactions of *p*-nitrophenyl hexanoate and, to a lesser extent, the acetate, with the anionic nucleophilic reagents hydroxide ion and leucine, but not with the uncharged reagent morpholine, are accelerated by CTAB. These assumptions also account for the inhibition of reaction of the esters with hydroxide ion and leucine by NaLS and NID and are consistent with the detergent inhibition of the reactions with morpholine.

The rate effects observed with leucine and morpholine in the presence of detergents is almost certainly not due to alteration of the pK_a of the conjugate acids of these species since (a) observed pH meter readings employing leucine and morpholine buffers were not altered in the presence of any of the detergents, (b) the qualitative effects of the reactions of leucine were similar to those for the reactions with hydroxide ion, and (c) the qualitatively dissimilar kinetic effects obtained in the presence of CTAB and NID are difficult to account for on this basis since the presence of these detergents affects the pK_a of the conjugate acid of *p*-chlorobenzylidene-1,1-dimethylethylamine in a qualitatively similar fashion.[3]

The above conclusions are consistent with the earlier observations that the basic hydrolysis of monolayers of octadecyl acetate is catalyzed by incorporation of trimethyloctadecylammonium ions into the surface[21] and that, for alkyl sulfates, the acid-catalyzed hydrolysis is accelerated and the base-catalyzed hydrolysis is inhibited by micellation.[22] Hydrolysis rates for ethyl benzoate, diethyl phthalate, and benzocaine are also known to be inhibited in the presence of certain detergents.[23,24]

Recently, Richards and co-workers have reported the effects of detergents on the rates of some related reactions.[25] In general agreement with the data reported here, these workers have established that the rate of the reaction of the zwitterionic species glycylglycine with 1-fluoro-2,4-dinitrobenzene is increased in the presence of CTAB but inhibited in the presence of NaLS. The rate of reaction of this reagent with glycinamide is also increased in the presence of CTAB but is unaffected by the presence of NaLS. These workers have also established the interesting result that for some bimolecular reactions the second-order rate constants are unaltered in the presence of detergent despite the fact that one reagent is incorporated into or onto the micelles while the other reagent is excluded from the micellar phase.

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