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DIPHENYL PHOSPHONATE ESTER CLEAVAGE CATALYZED BY HYDROPHOBIC AMMONIUM IONS

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Abstract. - The basic hydrolysis of diphenyl [1-(N-benzyloxycarbonylalanyl)-3-methyl]butylphosphonate have been studied in various cationic micelles. The kinetics of the reactions in buffered solutions and dilute sodium hydroxide solutions are compared.

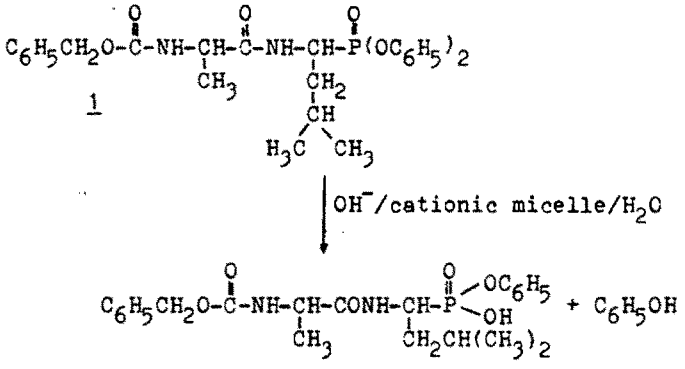
INTRODUCTION

During the past two decades there has been considerable interest in reactions which can be carried out at interfaces. Among them, the catalysis and inhibition by submicroscopic entities, such as micelles, have attracted increasing attention. Numerous surfactants have been used to catalyze alkaline hydrolysis of phosphorus esters[1-2] and most of these have been quaternary ammonium systems. The effect of cationic micelles on the rates of these bimolecular reactions is due to increased concentration of phosphorus esters and hydroxide ions in the small volume of the micellar Stern layer[3]. Simple electrostatic considerations predict that these micelles will enhance reaction rates.

Generally rate constants in micelles are similar to those in water and the differences are due to both properties of micelles as kinetic solvent and the different location of the two reactants in micelles[3].

Recently we have been engaged in the preparation of phosphono peptides in which (aminoalkyl)phosphonic acid is introduced at C-terminus of the peptide molecule. The vital problem of these syntheses is a selective removal of the blocking groups[4], and alkaline hydrolysis of diphenyl phosphonates to correspon-

ding monophenyl esters[5] is one of the most promising methods. In this paper we have studied the kinetics of the basic hydrolysis of totally blocked phosphono dipeptide - diphenyl [1-(N-benzyloxycarbonylalanylamino)-3-methyl]butylphosphonate 1, in various cationic micelles (Scheme I)



Scheme I

DISCUSSION

To compare the abilities of various hydrophobic quaternary ammonium salts to catalyze the basic hydrolysis of phenyl ester 1 the experiments were carried out at 20° C and pH 8.0 (0.2 molar phosphate buffer). The observed pseudo-first order rate constants, k_ψ, follow a typical pattern passing through maxima with increasing surfactant concentration[6-8]. The k_ψ values determined upon the concentration of surfactants yielding maximal catalytic activities are summarized in Table I.

Table I. Hydrolyses of peptide 1 in 0.2 M phosphate buffer

Surfactant	surfactant concentration [mM]	$10^5\ k_\psi\ [s^{-1}]$		
		sole surfactant	surfactant + 50mM KCl	surfactant + 50mM KF
CBDAC	2.5	4.4	2.7	16.0
	5.0	6.0	4.9	24.3
Cetrimide C_{16}	2.5	4.9	2.5	24.0
	5.0	4.5	3.8	19.7
Benzalkonium chloride C_{14}	2.5	5.7	2.2	12.7
	5.0	4.0	2.2	17.3
N-Cetylpyridinium chloride	2.5	8.8	12.2	31.3
	5.0	7.8	4.9	39.8
Scheroquat IIb	5.0	8.5	n.d.	n.d.
Scheroquat IIs	2.5	5.2	n.d.	8.2
	5.0	4.8	4.8	10.3
Amphoram BA 30	5.0	n.d	n.d.	5.2

n.d. = not determined because of turbidity

These rate constants are similar despite of the surfactant used being about 5×10^{-5} s^{-1}. The relatively low values of k_ψ resulted from the presence of high concentration of phosphate buffer ions, which compete with hydroxide ions for the vicinity of the quaternary nitrogens in the micellar Stern layer, yielding the decrease of the hydroxide ion to peptide 1 concentration ratio in the micellar environment.

Indeed the decrease of buffer concentration from 0.2 M to 0.02 M resulted in the significant increase of the k_ψ values. For example, the rate constants of the reaction carried out in the presence of 2.5 mM Cetrimide C_{16} or Benzalkonium chloride C_{14} are about 100-fold higher in 0.02 M (k_ψ values are $2.3 \cdot 10^{-3} s^{-1}$ and $3.4 \cdot 10^{-3} s^{-1}$ respectively) than in 0.2 M phosphate buffer (k_ψ values being $4.9 \cdot 10^{-5} s^{-1}$ and $5.7 \cdot 10^{-5} s^{-1}$ respectively). Also the use of 0.2 M borate buffer (pH 8) instead of 0.2 M phosphate buffer in the presence of 2.5 mM CBDAC resulted in elevated k_ψ value ($2.8 \cdot 10^{-4} s^{-1}$ and $4.4 \cdot 10^{-5} s^{-1}$ respectively).

These micellar effects represent a vivid ability of cationic micelles to concentrate anionic reagents relative to the aqueous pseudophase[9-10].

This ability was also confirmed by the addition of the excess of potassium chloride to the reaction medium. The unreactive chloride ions, additional negatively-charged species in the solution, also decrease the concentration of hydroxide ion in Stern layer, resulting in slight slowering of the hydrolysis (Table I). The only exception is the reaction catalyzed by N-Cetylpyridinium chloride. In this case the slight increase of the reaction rate constants upon addition of potassium chloride was observed. It indicates that effect of microenvironment is here predominant over the effect of high local concentration of hydroxide ion.

The hydrolysis of aromatic esters of phosphorus is speeded by the action of fluoride ions. The limiting step of the reaction is the formation of phosphorus--fluorine bond, which is then rapidly hydrolyzed by base. This reaction is also effectively catalyzed by hydrophobic quaternary ammonium salts[11-13]. Thus, the micellar reaction of dipeptide 1 with hydroxide ions is speeded by the presence of high concentration of fluoride ion (Table I). The observed k_ψ values are about 10-fold higher than those found in the absence of fluoride ions.

The hydrolysis of diphenyl [1-(N-benzyloxycarbonylalanylamino)-3-methyl]butylphosphonate in dilute aqueous solutions of sodium hydroxide is much faster than in buffered solutions (Table II). The order of reaction is dependent on the hydroxide ion to peptide 1 concentration ratio. If the 4-fold excess of peptide was used the reaction appeared to be of the first order, while the rate constants are comparable to those observed in 0.2 M phosphate buffer. This indicate that for the experiments summarized in Table I, the presence of high concentration

of phosphate ion strongly reduced the concentration of hydroxide ion in Stern layer.

Table II. Micellar hydrolyses of peptide 1 in dilute sodium hydroxide solutions

Concentration of NaOH [mM]	pseudo-first order reaction rate constant, $k_\psi \cdot 10^5 s^{-1}$		second order reaction constant k $[s^{-1} M^{-1}]$	
	Benzalkonium chloride C_{14} [a]	Cetrimide C_{16} [a]	Benzalkonium chloride C_{14} [a]	Cetrimide C_{16} [a]
0.05	1.3	0.8	-	-
0.1	-	-	15.0	8.1
0.2	-	-	8.1	9.1
0.3	-	227	11.7	-
0.4	610	357	-	-

[a] surfactant concentration 5mM

The first-order reaction was also observed if the reactions were carried out in the presence of 2-fold excess of hydroxide ion, and the observed k_ψ values are 100-fold higher than those observed in buffered solutions (Table II).

For intermediate peptide 1 to hydroxide ion concentration ratios the reaction is of the second order. It is worth noting that for the concentration of the base half of that of the peptide the reaction was finished when the half of the peptide was converted into monoester.

Our results suggest that all the peptide is solubilized in the micelles, when the effective concentration of hydroxide ion in Stern layer of the micelles is high enough to bring the substrates into close proximity and assist the reaction to completness.

EXPERIMENTAL

The surfactants: Cetrimide C_{16} (Cetyltrimethylammonium bromide) and Benzalkonium chloride C_{14} (Myristyldimethylbenzylammonium chloride; both from Danchemo A/S, Denmark), CBDAC (Cetyldimethylbenzylammonium chloride, from Fluka), N-Cetylpyridinium chloride monohydrate (Merck), Scheroquat IIa (2-isoheptadecyl-1-hydrogenethyl-1-ethyl imidazolinium ethyl sulfate) and Scheroquat IIb (2-isoheptadecyl--1-hydroxyethyl-1-benzyl imidazolinium chloride, both from Scher Chemicals Inc., New Jersey, U.S.A.) , and Amphoram BA 30 (N-cocodiethylbetaine, from CECA S.A., France), were used without purification.

Diphenyl [1-(N-benzyloxycarbonylalanylamino)-3-methyl]butylphosphonate 1.
This was prepared as described earlier[14], starting from carbobenzoxy-L-alanine and diphenyl (1-amino-3-methyl)butylphosphonate, as dense oil, in 83% yield:

IR (CCl_4): ν=3260 (NH); 1705 and 1650 (CO); 1520 (NH); 1245 (PO); 945 (POC)cm^{-1}; ^{1}H-NMR ($CDCl_3$/HMDS): δ=0.78 (bd, ^3J=7.5Hz,6H,$CH(C\underline{H}_3)_2$); 1.12 and 1.16 (d, ^3J=6.5Hz, 1.5H,$CHC\underline{H}_3$); 1.4 - 1.9 (m,3H,$C\underline{H}_2C\underline{H}$); 3.97 (q-q, ^3J=7.5Hz, ^3J=7.5Hz,1H,$C\underline{H}CO$); 4.17(t-t, ^3J=7.5Hz, ^2J=14.5Hz,1H,CHP); 4.98(s,2H,CH_2O); 5.45-6.25 (m,1H,NH); 7.00 and 7.04 (s,2.5H,$POC_6\underline{H}_5$); 7.10(s,5H,$POC_6\underline{H}_5$); 7.19(s,5H, $C_6\underline{H}_5CH_2$); 7.80 (bd, ^3J=7.5Hz,1H,NH). $[\alpha]_{20}^{578}$ = -17.5°±0.5° (c2, methanol). Elemental analyses calcd. for $C_{28}H_{33}N_2O_6P$ (524.5): 5.90% P and 5.34% N; found 6.20% P and 5.01% N.

Kinetic studies

Runs were performed at 20° C on Specord UV-VIS M40 Spectrophotomether in pH 8 (0.2 M phosphate or 0.2 M borate buffers) in a total volume of 2 ml. The mixture included: the surfactant (2.5 mM or 5.0 mM final concentration), potassium chloride or potassium fluoride (0 mM or 50 mM final concentration) and 50 μL of peptide 1 solution in acetonitrile (final concentration -0.2 mM). The appearance of phenolate was monitored at 270 nm (λmax). Values of pseudofirst order and second order rate constants were obtained graphically.

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