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Reactivity of Phosphorus Esters in Supramolecular Systems Based on Surfactants Containing an Uracil Residue and Polyethylenimine

D. R. Gabdrakhmanov, F. G. Valeeva, L. Ya. Zakharova, R. Kh. Giniyatullin, V. E. Semenov, V. S. Reznik, and A. I. Konovalov

Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center, Russian Academy of Sciences, ul. Arbuzova 8, Kazan, 420088 Tatarstan, Russia
e-mail: lucia@iopc.ru

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Abstract—The reactivity of phosphorus esters with different hydrophobicities was studied in aqueous solutions of cationic surfactants containing an uracil residue, as well as in binary systems based on polyethylenimine. Pronounced substrate specificity was revealed in all supramolecular systems examined; in particular, acceleration of the hydrolysis of more hydrophobic substrate and inhibition of the reaction with less hydrophobic analog were observed. Aggregation in the examined systems was confirmed by tensiometric and conductometric measurements. The aggregation threshold considerably decreased in going from monocationic amphiphile to more hydrophobic dicationic analog due to the presence in the latter of two additional alkyl radicals.

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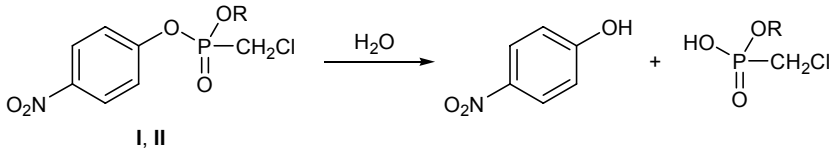
Reactivity control constitutes one of the most important problems of organic chemistry. Among numerous catalytic systems, a particular place is occupied by catalysis of chemical reactions in organized media based on amphiphilic compounds; such systems may be regarded as biomimetic, and they make it possible to simulate factors responsible for the reactivity of substrates in biological media [1, 2]. Two main factors are generally distinguished for catalytic reactions in synthetic supramolecular systems and enzymatic catalysis: (1) concentrating of the reactants (cage effect) and (2) change of their microenvironment (effect of the medium). These factors originate from the capability of amphiphilic compounds for self-organization and transfer of the reactants from the bulk solution to aggregates [3, 4]. Although catalytic reactions in organized media have been extensively studied [4–6], only a few examples of selective acceleration of reactions with structurally related substrates, i.e., substrate specificity typical of enzymatic catalysis, have been reported.

We previously described various supramolecular catalytic systems based on conventional surfactants (micelles and microemulsions) and macrocycles (calixarenes and polymers) and mixed amphiphile–polymer systems, which showed a high catalytic or inhibitory activity in nucleophilic substitution reactions with phosphorus esters [4, 7–9]. Phosphoryl group transfer is the key biochemical reaction in metabolic processes [10]. The applied aspect of relevant studies is equally important; for example, environmentally hazardous toxic phosphorus esters are decomposed by hydrolysis [11, 12]. Cationic surfactants are capable of accelerating alkaline hydrolysis of esters by enhancing the concentration of hydroxide ions on the surface of cationic micelles.

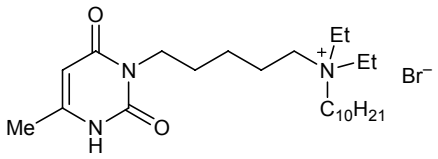
In the present work we studied the hydrolysis of two *p*-nitrophenyl alkyl chloromethylphosphonates **I** and **II** with different lengths of the alkyl radical (R = Et, C_6H_{13}) in aqueous solutions of acyclic surfactants APB-1 and APB-2 containing uracil fragments and bromide ions as counterions (Scheme 1). We previously reported [13–15] on the synthesis and aggregation properties of acyclic and macrocyclic amphiphiles containing pyrimidine fragments; these compounds were shown to constitute a new class of cationic surfactants possessing specific properties that differ from the properties of classical surfactants. Introduc-

Scheme 1.

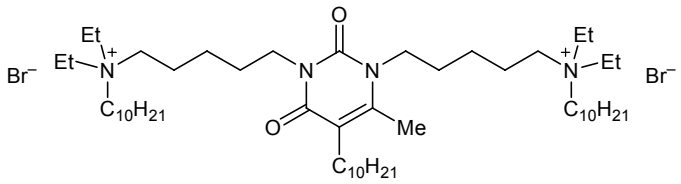


I, **II**

I, R = Et; **II**, R = C_6H_{13}.



APB-1



APB-2

tion into an amphiphile molecule of a 6-methyluracil fragment structurally related to the nucleobase uracil was expected to further highlight the biomimetic aspect of these studies and provide the possibility for the design of nanocontainers for targeted DNA delivery.

The formation of aggregates in solutions of APB-1 and APB-2 was confirmed by tensiometry and conductometry. Apart from surfactant solutions, we examined the system surfactant–polyethylenimine (PEI). The use of polymers allows one to replace micellar catalysts on the basis of non-covalently bound aggregates by immobilized nanoreactors. In addition, amino groups in polyethylenimine may enhance micellar catalysis factors due to the contribution of homogeneous catalytic mechanisms (base catalysis). The catalytic effect of polyethylenimine in the hydrolysis of phosphonates **I** and **II** was studied in [7].

Aggregation behavior. The reactivity of compounds in micellar surfactant solution is determined by aggregation [2–5]. We have studied those properties of the systems that are responsible for their catalytic effect. In particular, the critical micelle concentrations (CMC) and the degrees of counterion binding (β) were determined. The critical micelle concentration is the concentration of a surfactant corresponding to formation of micelles which act as nanosized reactors, and the degree of counterion binding determines the surface charge of aggregates and hence the concentration of nucleophile (hydroxide ion) in the reaction zone.

The tensiometric and conductometric data are presented in Figs. 1 and 2 as concentration dependences of the surface tension γ and electrical conductivity χ of aqueous surfactant solutions. These dependences have a bend at a definite surfactant concentration which is referred to as CMC. Two critical concentrations were found for APB-1, 3.0 and 10.0 mM, whereas one bend was observed for APB-2 at a concentration of 0.045 mM. The sharp decrease of CMC in going from the monocationic surfactant to dicationic analog may be rationalized by considerably increased hydrophobicity of APB-2 molecules possessing three decyl radicals. Comparison of the data for APB-2 and previously studied amphiphile containing a pyrimidine residue and two decyl radicals at the ammonium head groups [9] showed that the key factor is the presence of a hydrophobic substituent in the pyrimidine fragment, which strongly enhances the aggregation ability (the CMC decreases from 3 to 0.045 mM). We previously substantiated the assumption [13] that increase in the aggregation ability is determined not only by increased hydrophobicity of the surfactant due to the presence of an additional lipophilic substituent but also by the pos-

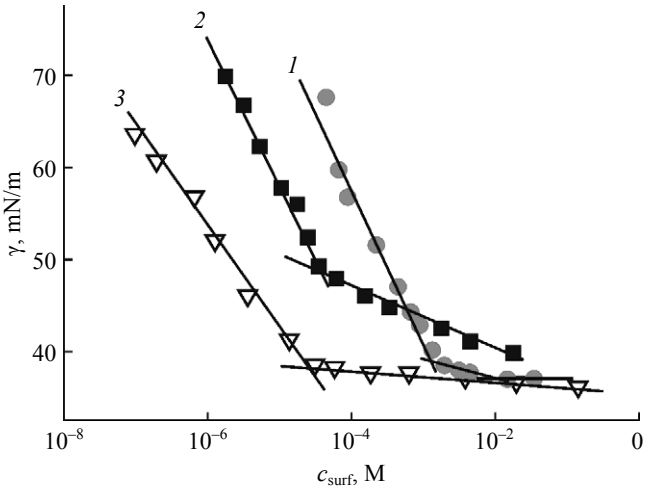


Fig. 1. Surface tension isotherms of aqueous solutions of (*1*) APB-1 and (*2*) APB-2 and (*3*) of binary system APB-2–polyethylenimine; c_{PEI} = 0.05 M, 25°C.

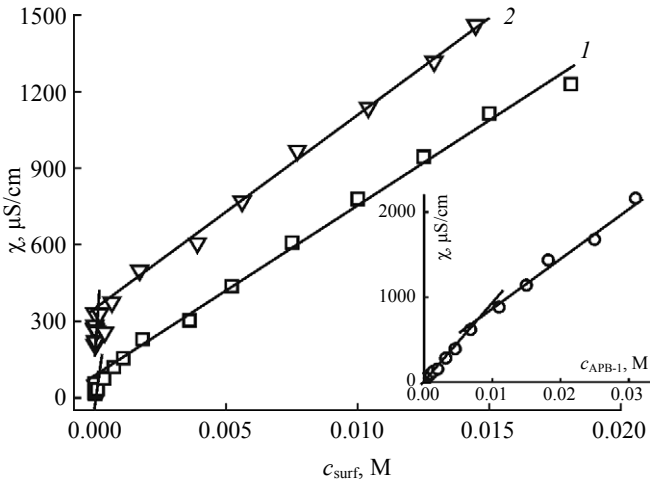


Fig. 2. Plots of the specific electrical conductivity of (*1*) aqueous solution of APB-2 and (*2*) binary system APB-2–polyethylenimine versus surfactant concentration; c_{PEI} = 0.05 M, 25°C. The corresponding dependence for APB-1 is shown in the insert.

sibility for more compact molecular packing. The data for the APB-2–polyethylenimine system (Fig. 1) show that addition of a polymer reduces the CMC even more strongly.

By quantitative analysis of the surface tension isotherms we calculated parameters characterizing micelle formation by the surfactants and adsorption at the water–air interface. The data in Table 1 show a good agreement between the trends in the variation of CMC and free energy of micelle formation in the examined systems. The conductometric data (Fig. 2) are consistent with those obtained by tensiometry; they confirm the CMC value of APB-1 close to 10 mM (Table 1).

One of the most important characteristics of ionic surfactants is the degree of counterion binding. By potentiometric measurements with the aid of a bromide-selective electrode we estimated the β value of APB-2 at 0.65–0.90; it decreases as the surfactant concentration increases.

Catalytic activity. In the absence of a surfactant, alkaline hydrolysis of phosphorus esters (Scheme 1) follows S_N2 (P) mechanism [16] with second-order rate constants of 4.0 and 3.0 L mol^{-1} s^{-1} for phosphonates **I** and **II**, respectively. Addition of surfactants does not change the reaction mechanism, and cationic micelles generally accelerate the process [4]. Figures 3 and 4 show the kinetic data for alkaline hydrolysis of phosphonates **I** and **II** in APB-1 and APB-2 micellar systems. The hydrolysis of phosphonate **II** was accelerated in both systems, by 3.8 and 27.7 times for micellar solutions of APB-1 and APB-2, respectively. This acceleration is determined by the concentration factor: the substrate is solubilized by the aggregates, and hydroxide ions bind to positively charged micelle surface via electrostatic forces.

Phosphonate **I** displayed an anomalous micellar effect: the rate of its hydrolysis decreased in APB-1 solution, while APB-2 did not affect the reaction rate. Rare examples of such substrate specificity, including inversion of the catalysis/inhibition effect, were noted in our earlier publications [17], in particular for surfactants containing a pyrimidine fragment. The following reasons for the anomalous micellar effect were presumed: (1) spontaneous acidification of the reaction solution; (2) steric hindrances to the attack by nucleophile; (3) low reagent–micelle binding constant. Our experiments showed that in the absence of alkali all solutions retained pH ~7 throughout the examined range of surfactant concentrations.

In order to estimate the substrate binding constants we analyzed the kinetic data in terms of the pseudophase model using Eq. (1), which is widely used in micellar catalysis [2]. This model implies formation of a substrate–micelle catalytic complex.

$$k_{obs} = \frac{k_w + k_{cat} K'_S c_{surf}}{1 + K'_S c_{surf}}. \quad (1)$$

Here, k_{obs} is the observed pseudofirst-order rate constant, k_w and k_{cat} are the first-order rate constants in water and catalytic complex, respectively, K'_S (L/mol) is the reduced micelle–substrate binding constant, and c_{surf} (mol/L) is the surfactant concentration.

Table 1. Maximum surface excesses (Γ_{max}), minimum surface areas per surfactant molecule (A_{min}), surface pressures (π_{CMC}), free energies of micelle formation (ΔG_m), and standard free energies of adsorption (ΔG_{ad}) for surfactant systems

System	CMC, M	$\Gamma_{max} \times 10^7$, mol/m^2	$A_{min.}$, nm^2	π_{CMC}, mN m^{-1}	$-\Delta G_m$, kJ/mol	$-\Delta G_{ad}$, kJ/mol
APB-1	3.0, 10.0	8.16	2.03	32.93	25.8	62.4
APB-2	0.045	5.62	2.96	21.7	31.3	62.7
APB-2–polyethylenimine	0.027	6.09	2.73	31.43	48.3	100

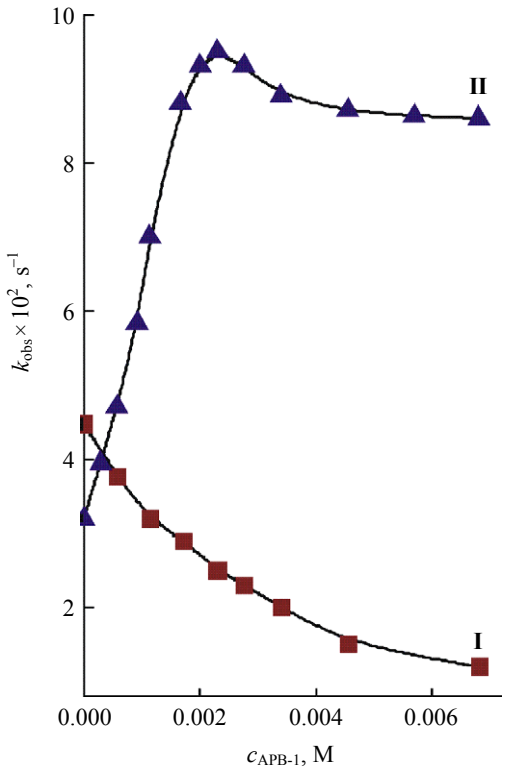


Fig. 3. Plots of the observed rate constant of alkaline hydrolysis of phosphonates **I** and **II** versus concentration of APB-1; 0.01 M NaOH, 25°C.

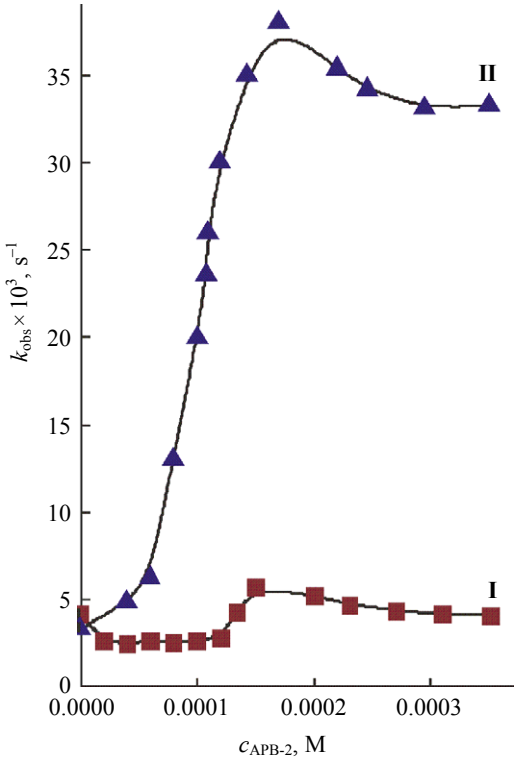


Fig. 4. Plots of the observed rate constant of alkaline hydrolysis of phosphonates **I** and **II** versus concentration of APB-2; 0.001 M NaOH, 25°C.

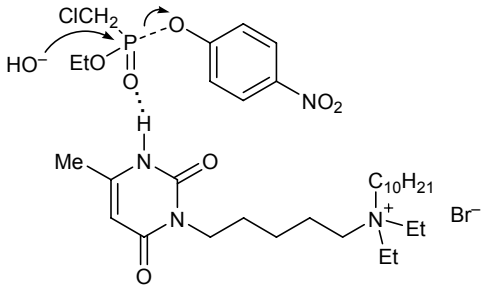
It is seen from the data in Table 2 that the binding constants of phosphonates **I** and **II** in both systems are comparable. Therefore, the concentrating effect cannot be responsible for the different effects of APB-1 and APB-2 on the reactivity of substrates differing by their hydrophobicity, and the observed substrate specificity is likely to be controlled by microenvironment of the phosphonates in micelles. This assumption may be regarded as fairly reasonable since molecules of phosphonates **I** and **II**, due to their different hydrophobicities, may be localized at different distances from the aggregate surface. Phosphonate **I** is likely to reside in the polar Stern layer, and more hydrophobic phosphonate **II**, in nonpolar micelle core. Furthermore, spatial proximity of phosphonate **I** molecules to the surface layer favors formation of hydrogen bond between the NH hydrogen atom of uracil and phosphoryl oxygen atom (Scheme 2), which could weaken polarization of the phosphoryl group and reduce the electrophilicity of the phosphorus atom and thus inhibit alkaline hydrolysis in the presence of APB-1.

Comparison of the micellar effects of APB-1 and APB-2 shows that the latter is a more efficient catalyst which selectively accelerates alkaline hydrolysis of

Table 2. Results of quantitative analysis of the kinetic data for the hydrolysis of phosphonates **I** and **II** in surfactant-based systems with the use of Eq. (1)

System	Phosphonate	$k_{cat} \times 10^3$, s^{-1}	K_S, L/mol	k_{cat}/k_w
APB-1	**I**	0.3	369	0.01
	II	121.7	499	3.80
APB-2	**I**	4.1	7059	1.00
	II	90.6	1496	27.7
APB-1–polyethylenimine	**I**	0.4	1022	0.30
	II	3.7	424	4.5 (10.0)

Scheme 2.



phosphonate **II** by a factor of ~30, whereas the reactivity of less hydrophobic analog **I** remains unchanged. It is important that the micellar effect of APB-2 is observed at a considerably lower concentration (μM level) as compared to APB-1. In keeping with the data in Table 2, the higher catalytic activity of APB-2 is related to the higher micelle–substrate binding constants which exceed those observed for APB-1 by a factor of 4–5.

Immobilization of micelles on a polymer matrix may enhance the catalytic efficiency of APB-1 and facilitate separation of the catalyst (which is important from the practical viewpoint). The data in Table 2 show that the system APB-1–PEI accelerates alkaline hydrolysis of phosphonate **II** by an order of magnitude relative to the rate of hydrolysis in the absence of surfactant, the high substrate specificity being retained. In fact, the alkaline hydrolysis of phosphonate **I** slows down in going from aqueous solution to both APB-1 solution and ABP-1–PEI binary system.

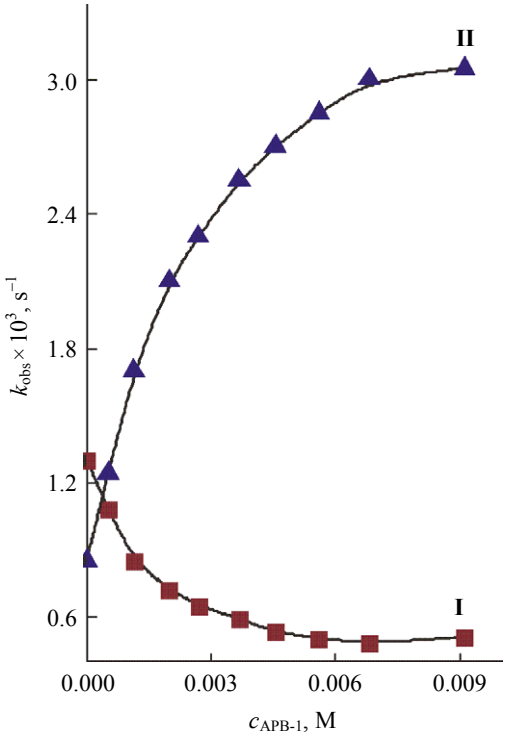


Fig. 5. Plots of the observed rate constant of alkaline hydrolysis of phosphonates **I** and **II** versus concentration of APB-1 in the binary system APB-1–polyethylenimine, c_{PEI} = 0.05 M, 25°C.

To conclude, our study of the aggregation behavior and catalytic activity of new amphiphiles containing an uracil fragment, taken alone and in a combination with PEI, revealed a stepwise reduction of the critical micelle concentration in the series monocationic surfactant > dicationic surfactant > binary system dicationic surfactant–polyethylenimine. The supramolecular systems thus formed considerably affect the reactivity of alkyl *p*-nitrophenyl chloromethylphosphonates, so that the rate of their hydrolysis can be varied over a wide range (from inhibition to acceleration). All catalytic systems examined displayed high substrate specificity: they selectively accelerated the hydrolysis of more hydrophobic phosphonate **II** at low surfactant concentrations.

EXPERIMENTAL

Phosphonates **I** and **II** were synthesized according to the procedure described in [18]. Surfactants APB-1 and APB-2 were prepared as reported [15]. Branched polyethylenimine (*M* 25000, Aldrich) was used; its molar concentrations are given with respect to the monomer unit.

The kinetic measurements were performed under pseudofirst-order conditions by spectrophotometry using a Specord UV-Vis spectrophotometer; the absorbance of *p*-nitrophenoxide ion was monitored at λ 400 nm. The observed rate constants (k_{obs}) were calculated by Eq. (2):

$$\ln(A_\infty - A) = -k_{obs}\tau + \text{const.} \quad (2)$$

Here, A and A_∞ are, respectively, the optical densities of reaction solution at a time τ and by the end of the process. The data were processed according to the weighted least-squares; mean values from three parallel measurements differing by no more than 5% were taken.

The surface tension was measured at 25°C by the du Noüy ring detachment method with the aid of a Krüss K6 tensiometer.

The maximum surface excess Γ_{max} was calculated from the Gibbs adsorption equation (3):

$$\Gamma_{max} = \frac{1}{2.3nRT} \lim_{c \to CMC}(\partial\pi/\partial \log c), \quad (3)$$

Here, π is the surface pressure equal to the difference in the surface tensions of a pure solvent and a solution with a given surfactant concentration ($\pi = \gamma_0 - \gamma$). The constant n is equal to 2 for ionic surfactants consisting of a singly charged micelle-forming ion and counterion, and $n = 3$ for dimeric surfactants consisting of a doubly charged micelle-forming ion and two singly charged counterions.

The minimum surface area per surfactant molecule (A_{min}), the free energy of micelle formation (ΔG_m), and the standard free energy of adsorption (ΔG_{ad}) were calculated by formulas (4) and (5):

$$A_{min} = \frac{10^{18}}{N\Gamma_{max}}; \quad (4)$$

$$\Delta G_m = (1 + \beta)RT\ln(CMC), \quad (5)$$

where N is the Avogadro number, and β is the degree of counterion binding.

The specific electrical conductivity was measured using an Inolab conductometer (Germany). The concentration of free bromide ions was determined with the aid of an I-160MI ionometer using an ELIS-131Br bromide-selective electrode and an ESR-10101 reference electrode. The concentration of bromide ions was calculated from the known Nernst equation (6) which relates the electrode potential (ΔE) to the activity of bromide ions (a_{Br^-}):

$$\Delta E = -\frac{RT}{F} \log(a_{Br^-}) + \text{const}. \quad (6)$$

Here, R is the universal gas constant, T is the temperature, and F is the Faraday constant; in an ideal case, the slope of this dependence $RT/F = 59.2$ mV× equiv^{-1} at 298.2 K.

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