Effects of Molecular Association on the Rates of Hydrolysis of Long-Chain Alkyl Betainates (Alkoxycarbonyl-*N,N,N*-Trialkylmethanaminium Halides)

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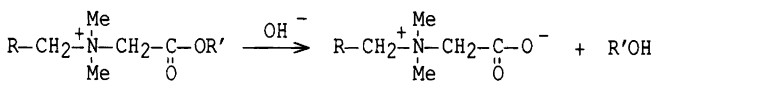
Alkaline hydrolysis of long-chain alkyl betainates (alkoxycarbonyl-*N,N,N*-trialkylmethanaminium halides) is accelerated by molecular aggregation leading to micellization. With increasing length of the alkyl chain, the observed rate constant shows an increased concentration dependence and an increased deviation from pseudo first-order kinetics at constant pH. The rate constant reached a maximum at an ester concentration close to the estimated critical micelle concentration. Thus, at a ca. 400 μM ester concentration the C_{14} compound was hydrolyzed roughly ten times faster than its C_3 analogue, while extrapolation to zero concentration gave virtually identical rate constants. The results are best interpreted by means of the pseudophase ion-exchange model.

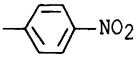
Long-chain alkyl betainates (alkoxycarbonyl-*N,N,N*-trimethylmethanaminium chlorides: type **1** compounds, Scheme 1) are cationic amphiphilic compounds with antimicrobial activity against a wide range of microorganisms.[1] They are primarily active against bacteria and microscopic fungi, but activity has also been shown against certain viruses such as *Herpes simplex,* HIV and to some extent papovavirus, as well as mammalian cells.[1]

It has been known since the 1950s that the short-chain alkyl esters (methyl and ethyl) of betaine are subject to an exceptionally rapid alkaline hydrolysis[2-4] (Scheme 1), whereas the rate of acid hydrolysis is considerably slower than in normal esters.[2,5] The reason for this behaviour is found in the destabilization of the ground state of the ester due to the positive charge at the nitrogen atom. This causes an unfavourable electrostatic effect (charge repulsion involving the carbonyl carbon atom), which is relieved by hydroxide ion attack, but potentiated by protonation. The net result of this effect will be an unusually large pH-dependence of the rate of hydrolysis.

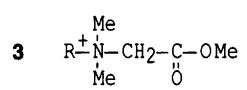
This type of kinetic behaviour was also found for a type **1** compound with the biologically interesting alkyl chain length of C_{14}.[1] The results of these experiments initiated further kinetic studies of alkyl esters of a betaine containing a chromophore label, type **2** compounds. The studies, which are presented in this paper, revealed a concentration-dependent mechanism. At or above the critical micelle concentration (cmc), deviation from first-order kinetics and a considerable rate enhancement with increasing chain length was observed. All our data indicate micellar catalysis as the primary cause of this increased reactivity.



1 R = H

2 R = 

R′ = C_3H_7 (a) $C_{10}H_{21}$ (b) $C_{12}H_{25}$ (c) $C_{14}H_{29}$ (d)



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Scheme 1.

Experimental

Synthesis. Compounds **1** were prepared via chloroacetylation of the appropriate alkanol followed by reaction of the resulting chloroacetate with trimethylamine.[1,6,7] Compounds **2** were obtained by first treating the chloroacetate with dimethylamine and then alkylating the tertiary amine function with 4-nitrobenzyl bromide. The synthesis of **2b** is representative of the procedure.

Decyl chloroacetate. 1-Decanol (19.4 g, 122 mmol) was dissolved in 100 ml of dichloromethane and chloroacetyl chloride (14.2 g, 126 mmol) in 25 ml of dichloromethane was added dropwise with stirring. The reaction mixture was stirred for 6 h and then subjected to gentle reflux for 0.5 h. The dichloromethane solution was washed with 5 % sodium hydrogen carbonate and water to remove excess chloroacetyl chloride, dried over magnesium sulfate, filtered and evaporated. The product weighed 27.7 g, corresponding to a 97 % yield.

Decyl N,N-*dimethylglycinate hydrochloride (decyloxy*-N,N-*dimethylmethanaminium chloride).* Dimethylamine (9.96 g, 221 mmol) was slowly bubbled into 150 ml of ether with stirring and cooling in an ice-bath. The chloroacetate (10.0 g, 42.6 mmol), dissolved in 100 ml of diethyl ether, was then added dropwise and the solution was allowed to attain room temperature. After 72 h, the precipitate, mainly dimethylamine hydrochloride, was removed by filtration and the ether was evaporated. The residue was dissolved in 100 ml of diethyl ether and washed with 100 ml of 10 mM sodium hydroxide solution. The ether solution was dried ($MgSO_4$), and the ester hydrochloride precipitated by the introduction of hydrogen chloride gas. The product was isolated on a glass filter, washed with dry ether and dried. The yield was 10.8 g (91 %).

Decyl N,N-*dimethyl*-N-*(4-nitrobenzyl)glycinate bromide (decyloxycarbonyl*-N-N-*dimethyl*-N-*(4-nitrobenzyl)methanaminium bromide).* The hydrochloride (5.0 g, 17.9 mmol), dissolved in 300 ml of 10 mM hydrochloric acid, was transferred to a separatory funnel containing 200 ml of diethyl ether and the aqueous phase was made basic by the addition of 25 ml of 2 M sodium hydroxide solution. The liberated free amino acid ester was further extracted with three 100 ml portions of diethyl ether. After drying of the combined ether extracts, 4-nitrobenzyl bromide (4.1 g, 19 mmol) was added and the reaction mixture was stirred for 24 h, the last 1 h at reflux temperature. The ether was then evaporated and the residue recrystallized from ethyl acetate – benzene (1:1). M.p. 115.5–6.0 °C. Yield 4.3 g (53 %). ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.32 (2 H, d, *J* 9.0 Hz, C_6H_4), 7.99 (2 H, d, *J* 9.0 Hz, C_6H_4), 5.64 (2 H, s, $ArCH_2$), 4.89 (2 H, s, CH_2CO), 4.20 (2 H, t, *J* 7.0 Hz, CH_2O), 3.61 [6 H, s, $N(CH_3)_2$], 1.60 (4 H, m), 1.27 (14 H, m), 0.88 (3 H, t, *J* 7.0 Hz).

All compounds were purified by recrystallization and were checked for purity by TLC or LC. Their identities were confirmed by NMR spectroscopy.

Kinetic investigations. GLC investigations. A stock solution of the ester was prepared in water at ten times the desired concentration. Test tubes containing 450 µl phosphate buffer solution were allowed to stand for 1 h in a water bath at the desired temperature. The hydrolysis was started by adding 50 µl of the ester solution to each test tube. Hydrolysis was terminated by the addition of 40 µl of 2 M HCl. The samples were extracted with 1.5 ml hexane containing 0.1 mg ml^{-1} hexadecanol followed by 1.5 ml hexane. The combined hexane phases were concentrated to 0.2 ml under a stream of nitrogen.

The gas chromatograph was an Antek model 464 LP (Antek, Houston, TX, USA) equipped with a flame ionization detector. The column was a RSL-150, 10 m×0.53 mm ID (Alltech, Deerfield, IL, USA). Nitrogen was used as the carrier gas at 10 psi. The column oven was programmed from 85 to 180 °C at 10° min^{-1}. Injections of 3 µl with a split of 1:2 were made. Retention times and peak areas were obtained by an electronic integrator (Waters model 740, Milford, MA, USA) interfaced with the detector.

HPLC investigations. Kinetics were followed as with the GLC method, except that, instead of extraction with hexane, the emulsion was broken by the addition of 1.5 ml acetonitrile. The solution (20 µl) was then injected onto the HPLC column.

The HPLC column was 2.6×200 mm and packed with Nucleosil 5 C-18 (Macherey–Nagel, Düren, FRG). The mobile phase consisted of water, adjusted to pH 2.9 with HCl, in acetonitrile, the optimal composition varied depending on the ester. Absorbance was measured at 257 nm.

The chromatographic system consisted of an LKB model 2150 pump (Bromma, Sweden), a Rheodyne 7125 sample injector (Cotati, CA, USA) equipped with a 20 µl loop, and an ISCO model V^4 variable wavelength absorbance detector (Lincoln, NE, USA).

Cmc determinations. The cmc values were determined in distilled water using a Philips model PW 9506 conductivity meter (Pye Unicam Ltd., Cambridge, UK). The specific conductance (mS cm^{-1}) values obtained for a series of ester concentrations at 25 °C were used to determine the cmc by plotting the equivalent conductance (S cm^2 mol^{-1}) against the square root of the ester molarity.[8]

Results and discussion

Reactions studied. Rate constants for compounds **1** were obtained by gas chromatographic (GLC) determination of the liberated alkanol as a function of time, whereas for

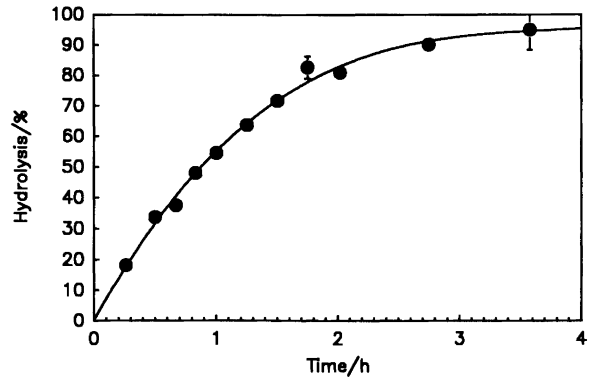

Fig. 1. Hydrolysis of compound **2d** (193 μM) in 100 mM phosphate buffer, pH = 8.0, at 25 °C.

compounds **2**, containing a chromophore label, the time-dependence of the ester concentration was determined by high-pressure liquid chromatography (HPLC). The main part of the investigation is based on the latter method since it gave more accurate data.

Kinetics. The alkaline hydrolysis of methyl betainate has been shown to be strictly second order, first order in betainate and hydroxide ion.[2-4] Therefore, at constant pH the reaction can be treated as pseudo first-order. Kinetic studies of the tetradecyl ester of betaine (**1d**) at pH 7.0 and 35 °C gave an observed (taken as pseudo first-order) rate constant $k_{obs} = 9.17\times10^{-5}$ s^{-1}, which is higher than that reported[4] for methyl betainate (3.06×10^{-5} s^{-1}, 25 °C, pH = 8.6). An investigation of the series **2a–d** was initiated which showed that the rate constants for the higher homologues in the series were dependent upon the initial concentration used. The time-dependence of the hydrolysis of **2d** (193 μM) at pH 8.0 and 25 °C is shown in Fig. 1. The use of a pseudo first-order equation [eqn. (1)] yields good linearity (Fig. 2), giving a half-life of 48 min.

$$k_{obs} = 1/t \ln a_0/a \quad (1)$$

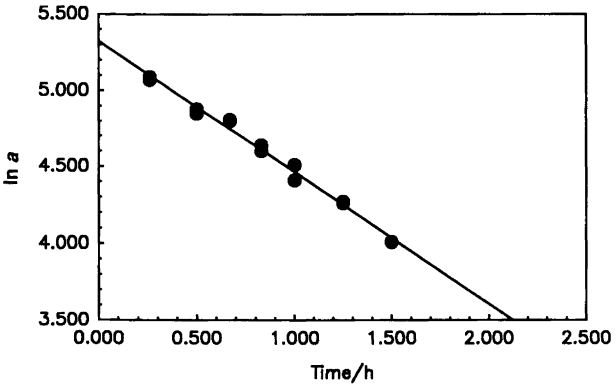

Fig. 2. Pseudo first-order plot of the hydrolysis of compound **2d** (conditions as in Fig. 1).

Table 1. The effect of the alkyl chain length in compounds **2** on the observed rate constant at 25 °C in 100 mM phosphate buffer, pH = 8.0.

Compound	Conc./μM	10^5 k_{obs}/s^{-1}
2a	800	3.44
2b	800	6.69
2c	800	20.8
2d	800	27.3

Table 1 gives the rate constants for **2a–d** at 25 °C and 800 μM initial concentration. The absence of a rate-retarding effect by the size of the alkyl chain is notable in view of the very large effect demonstrated for a series of 4-nitrophenyl alkanoates.[9] For the latter substances, the rate of hydrolysis was found to decrease by 102 on going from the acetate to the hexadecanoate. This effect was attributed to steric shielding of the ester carbonyl group due to the coiling of the alkyl chain.

Studies of compound **2d** over a wider range of concentrations revealed not only a marked concentration dependence, but also a reversed order of reactivity. Fig. 3 shows a plot of rate constants for **2a–d** approximated by the assumption of pseudo first-order reaction kinetics, versus concentration below and above the cmc. The cmc values for these compounds in deionized water listed in Table 2 give an approximate idea of the cmc in the buffer solution used in the hydrolysis studies.

Compounds of type **2** where R′ = $C_{16}H_{33}$ or $C_{18}H_{37}$ were also prepared. These were not included in the hydrolysis study because of their low cmc and solubility.

The results obtained for compounds **2a–d** indicate micellar catalysis and are in agreement with the pseudophase ion-exchange model.[10-12] The rapidly ascending branch of the curves in Fig. 3 can be accounted for by the formation of micelles, in which the ester bonds and the hydroxide ions are concentrated in the small pseudophase formed by the Stern layer. The descending branch of the curves above the cmc is assumed to be caused by displacement of hydroxide

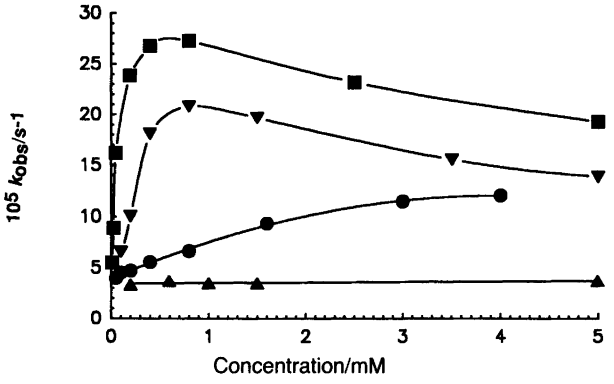

Fig. 3. Concentration dependence of k_{obs} for compounds **2a** (▲), **2b** (●), **2c** (▼), and **2d** (■). Measurements made at 25 °C in 100 mM phosphate buffer, pH = 8.0.

Table 2. Cmc for compounds **2b–d** in deionized water at 25 °C.

Compound	cmc/μM
2b	3860
2c	812
2d	149

ions by the increasing concentration of the unreactive bromide counter-ions.

Earlier studies of hydrolysis of long-chain betaine esters[13,14] have been performed with esters of type **3**, where R was an alkyl group in the range C_{10} to C_{16}. These compounds have the ester bond oriented in the opposite direction in the micelles as compared with **1** and **2**. The studies were, with one exception, carried out in the presence of a stable surfactant, e.g., cetyltrimethylammonium chloride (CTACl). The rate constants obtained could be accounted for by the pseudophase ion-exchange model. Al-Lohedan *et al.*[13] also studied a compound that could undergo self-micellization (**3**, R = $C_{16}H_{33}$). The rate constants for this compound increased with increasing concentration, reaching an asymptotic value. This can be compared to the ascending branch of the curves in Fig. 3.

Micellar effects upon the rate of reaction can be explained not only by the distribution of the reactants between the water and micellar pseudophases, but also by the second-order rate constants in each of these phases, k_w and k_m, respectively. It has been suggested[12] that the micellar pseudophase is an environment of moderate polarity similar to that of ethanol. Haberfield and Forter[15] found that a type **2** ester where R′ = C_2H_5 (ethoxycarbonyl-*N,N,N*-trimethylmethanaminium chloride) had a somewhat lower second-order rate constant in 60 % aqueous ethanol than in water. In general, it has been found that the second-order rate constants in the two pseudophases are similar, with $k_m/k_w \leq 1$.[12] For the type **3** compounds it was found that $k_m/k_w \approx 0.25$.[13,14] This can be understood in light of the high ionic concentration in the Stern layer and the negative salt effect on the alkaline hydrolysis of non-micelle forming betaine esters in water.[13] The type **2** betaine esters would thus be expected to have $k_m/k_w \leq 1$, and the micellar catalytic effect observed for these compounds must be a result of the increased concentration of the hydroxide ions in the micellar pseudophase.

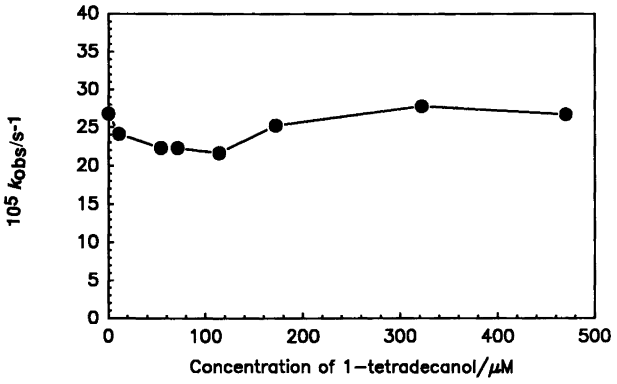

Fig. 4. Effect of the addition of 1-tetradecanol on the k_{obs} of **2d** (400 μM). Measurements made at 25 °C in 100 mM phosphate buffer, pH = 8.0.

Since the micelle-forming surfactant is also one of the reactants in the systems studied, a change in the micelle composition with time is to be expected. Hydrolysis of the ester will release a long-chain alcohol, which will form mixed micelles with the remaining ester. Fig. 4 shows the effect on the rate constant of the addition of 1-tetradecanol to a 400 μM solution of **2d**. It is apparent that the ratio of alcohol to ester affects the rate of hydrolysis only slightly.

Even if the formation of mixed micelles has only a small effect on the rate constants, the hydrolysis should proceed to a point where the concentration of the ester is below the cmc and the rate constant will decrease. As shown in Fig. 1 the curve does not indicate 100 % hydrolysis within the expected time. The variation in the data also increases. These effects suggest that only initial hydrolysis rates can be considered.

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