# Hydrolysis of *p*-Nitrophenyl Hexanoate in Hydrophobic Aggregate System

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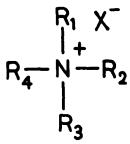
(Received December 5, 1985)

**Synopsis.** Hydrolysis of *p*-nitrophenyl hexanoate in the presence of azide ion ($N_3^-$) was accelerated by tetraheptylammonium bromide (**1**), which has four long alkyl chains. In contrast to **1**, the other tetraalkylammonium salts which have shorter alkyl chains, (**2**)—(**5**), did not elevate the reactivity. *p*-Nitrophenyl acetate, less hydrophobic substrate, was not hydrolyzed so efficiently even in the presence of **1**.

An enzyme holds a specific conformation in an aqueous solution, where hydrophilic groups are situated on the outer surface and hydrophobic groups are placed inside. As micelles resemble enzymes in this respect, lots of studies have been undertaken about micelle-system reactions from an enzyme-mimetic point of view.[1-6] In micelle systems substrates are incorporated into or adsorbed on micelles by electrostatic and/or hydrophobic interactions and activated in specific environments. Other enzyme-mimetic systems such as vesicles[7-9] and aggregate of methyltrioctylammonium chloride[10,11] are also studied intensively, where reactions are activated as compared with aggregate-free ones. In the present report, we describe the effect of a new type of hydrophobic aggregate made up of tetraheptylammonium bromide **1**, which possesses four long alkyl chains, upon the hydrolysis of an active ester (*p*-nitrophenyl hexanoate) in the presence of azide ion ($N_3^-$).



(1) $R_1=R_2=R_3=R_4=n\text{-}C_7H_{15}$, X=Br
(2) $R_1=R_2=R_3=R_4=n\text{-}C_6H_{13}$, X=I
(3) $R_1=R_2=R_3=R_4=n\text{-}C_5H_{11}$, X=Br
(4) $R_1=R_2=R_3=R_4=C_2H_5$, X=$ClO_4$
(5) $R_1=CH_2Ph$, $R_2=R_3=R_4=C_2H_5$, X=Br

## Experimental

Tetraheptylammonium bromide (**1**) and tetrapentylammonium bromide (**3**) (Aldrich Chemical Co.), tetrahexylammonium iodide (**2**) (Tokyo Kasei Kogyo Co.), tetraethylammonium perchlorate (**4**) (Nakarai Chemicals), benzyltriethylammonium bromide (**5**) (Kanto Chemical Co.), sodium azide (Nakarai Chemicals), *p*-nitrophenyl hexanoate (*p*-NPH) (Tokyo Kasei Kogyo Co.), *p*-nitrophenyl acetate (*p*-NPA)(Aldrich Chemical Co.), and 8-anilino-1-naphthalenesulfonic acid magnesium salt (ANS) (Nakarai Chemicals) were used as supplied.

The reaction was carried out at pH 8.0, $\mu$=0.5(KCl), buffered with 0.1 mol $dm^{-3}$ $KH_2PO_4$-0.05 mol $dm^{-3}$ $Na_2B_4O_7$. The reaction rate was determined by measuring the absorbance of liberated *p*-nitrophenol at 400 nm with a Beckman Model 34 spectrophotometer. Fluorescence intensity was measured under the same conditions as described above with a JASCO FP-4 fluorescence spectrophotometer.

## Results

The reaction rate did not follow pseudo-first-order even when $[N_3^-] \gg [p\text{-NPH}]$, that is, $\ln\{c_0/(c_0-x)\}$ was not proportional to the reaction time ($t$) and the rate constant (the slope of the curve) decreased with time. Here, $c_0$ is the concentration of *p*-NPH at a time 0 and $x$ is the concentration of *p*-nitrophenol at a time $t$. We have estimated the rate constant ($k$), therefore, from the slope of the curve at initial stage. On the other hand, the hydrolysis of *p*-NPH followed pseudo-first-order in the presence of $N_3^-$ and hexadecyltrimethylammonium chloride (HTAC) micelle.

In Fig. 1 are shown the relations between $k$ and the concentrations of the tetraalkylammonium salts ($C$), where $k$ increased monotonously with increasing the concentration of **1** in the presence of $N_3^-$ ($5\times10^{-4}$ mol $dm^{-3}$). In the absence of $N_3^-$, however, $k$ remained fairly small in spite of the addition of **1** ($5\times10^{-5}$ mol $dm^{-3}$). The $k$ value became more than 10 times larger upon addition of $N_3^-$ ($5\times10^{-4}$ mol $dm^{-3}$), when the concentration of **1** was $5\times10^{-5}$ mol $dm^{-3}$.

In the presence of **1** ($5\times10^{-5}$ mol $dm^{-3}$) $k$ increased with increasing $[N_3^-]$, whereas the $k$ value had a tendency to be saturated at higher $[N_3^-]$ (Fig. 2). When **1** was not present, $k$ increased little even if $N_3^-$ was added.

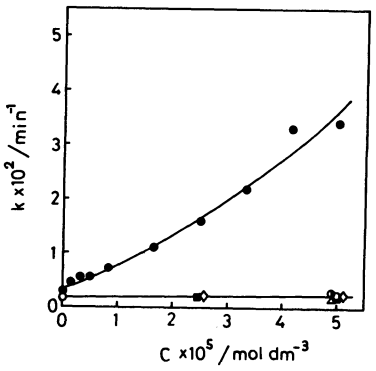



Fig. 1. Relationship between concentration of tetraalkylammonium ($C$) and rate constant ($k$) for hydrolysis of *p*-NPH in the presence of $N_3^-$; pH 8.0 (0.1 mol $dm^{-3}$ $KH_2PO_4$-0.05 mol $dm^{-3}$ $Na_2B_4O_7$ buffer solution), $\mu$=0.5(KCl), 25 °C, $[p\text{-NPH}]=5\times10^{-5}$ mol $dm^{-3}$, $[NaN_3]=5\times10^{-4}$ mol $dm^{-3}$.
(—●—): **1**, (—○—): **1**, $N_3^-$ free; (—■—): **2**, (—△—): **3**, (—◑—): **4**, (—◇—): **5**.

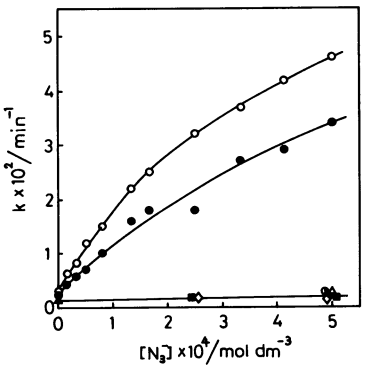



Fig. 2. Relationship between concentration of $N_3^-$ ($[N_3^-]$) and rate constant ($k$) for hydrolysis of $p$-NPH in the presence of $N_3^-$; pH 8.0 (0.1 mol dm$^{-3}$ $KH_2PO_4$ -0.05 mol dm$^{-3}$ $Na_2B_4O_7$ buffer solution), $\mu$=0.5 (KCl), 25 °C, [$p$-NPH]=5×10$^{-5}$ mol dm$^{-3}$, [Tetraalkylammonium]=5×10$^{-4}$ mol dm$^{-3}$. (—●—): **1**, (—○—): **1**, 29 °C, (—■—): **2**, (—▲—): **3**, (—◑—): **4**, (—◇—): **5**, (—△—): tetraalkylammonium free.

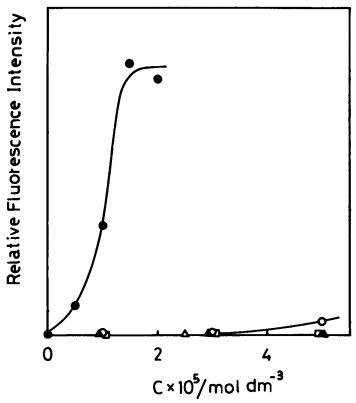



Fig. 3. Relationship between concentration of tetraalkylammonium ($C$) and relative fluorescence intensity of ANS; pH 8.0 (0.1 mol dm$^{-3}$ $KH_2PO_4$-0.05 mol dm$^{-3}$ $Na_2B_4O_7$ buffer solution), $\mu$=0.5(KCl), 25 °C, [ANS]=1×10$^{-4}$ mol dm$^{-3}$; excitation wavelength was 365 nm and emission wavelength was 490 nm. (—●—): **1**, (—○—): **2**, (—▲—): **3**, (—△—): **4**, (—□—) **5**.

Contrary to **1**, the other tetraalkylammonium salts, **2**—**5**, with less hydrophobic groups, did not accelerate the hydrolysis even in the presence of $N_3^-$ (Figs. 1 and 2). Furthermore, the hydrolysis of $p$-NPA (less hydrophobic substrate than $p$-NPH) was not enhanced under the same conditions as those for $p$-NPH.

It is generally known that the fluorescence intensity of ANS is significantly increased when ANS is incorporated into hydrophobic environment.[12] Therefore we studied the effect of the tetraalkylammonium salts upon the fluorescence of ANS to see which ammonium salts form hydrophobic aggregates (Fig. 3). Relative fluorescence intensity was remarkably increased in the presence of **1** (≦2×10$^{-5}$ mol dm$^{-3}$), while **2**—**5** scarcely increase the fluorescence.

## Discussion

It seems very likely that the hydrolysis proceeds as follows.

$$CH_3(CH_2)_4COOC_6H_4NO_2 + N_3^- \longrightarrow CH_3(CH_2)_4CON_3 + {}^-OC_6H_4NO_2 \quad (1)$$

$$CH_3(CH_2)_4CON_3 + OH^- \longrightarrow CH_3(CH_2)_4COOH + N_3^- \quad (2)$$

Therefore in the present study we are discussing the first reaction (1) of the hydrolysis.

As described in the Results section, the hydrolysis of $p$-NPH in the presence of $N_3^-$ and **1** did not follow pseudo-first-order plot. Taking into account the results that the hydrolysis was significantly accelerated upon addition of $N_3^-$, it seems very likely that $N_3^-$ adsorbed on the aggregate should attack $p$-NPH very efficiently. As the concentration of **1** (≦5×10$^{-5}$ mol dm$^{-3}$) is lower than that of $N_3^-$ (≦5×10$^{-4}$ mol dm$^{-3}$), the numer of $N_3^-$ adsorbed on the aggregate is appreciably decreased as the reaction proceeds, for the liberated $p$-nitrophenol and/or hexanoic acid can be also adsorbed on the anion-binding site of the aggregate in competition with $N_3^-$. Therefore $k$ will decrease as the hydrolysis proceeds. On the other hand, in the micelle system the concentration of HTAC (≧1×10$^{-3}$ mol dm$^{-3}$)was much higher than that of **1**, and so the number of $N_3^-$ on the micelle may be virtually constant. The $k$ value will be constant, therefore, during the reaction, as mentioned above.

In the presence of **1**, the $k$ value increased but seemed to be saturated gradually as $N_3^-$ was added (Fig. 2). This saturation may be attributed to the saturation of the amount of $N_3^-$ on the **1**-aggregate. When **2**—**5** were added instead of **1**, the reaction was not accelerated even in the presence of $N_3^-$ (Figs. 1 and 2). These results suggest that **2**—**5** cannot form aggregates in aqueous solution. This conclusion is also substantiated by the findings that the fluorescence intensity of ANS was remarkably increased by **1** but not by **2**—**5** (Fig. 3). An active ester, more hydrophilic than $p$-NPH, would not be included in **1**-aggregate. In fact, the hydrolysis of $p$-NPA was not activated in the presence of both **1** and $N_3^-$. Thus, it can be concluded that the rate enhancement of the hydrolysis of $p$-NPH in the presence of **1** and $N_3^-$ is ascribed to the formation of the aggregate of **1** on which $N_3^-$ and $p$-NPH are adsorbed and activated.

### References

1) Y. Murakami, A. Nakano, A. Yoshimatsu, and K. Matsumoto, *J. Am. Chem. Soc.*, **103**, 2750 (1981).
2) K. Ogino, I. Tomita, K. Machiya, and W. Tagaki, *Chem. Lett.*, **1982**, 1875.
3) C. A. Bunton, S. E. Nelson, and C. Quan, *J. Org. Chem.*, **47**, 1157 (1982).

4) H. Al-Lohedan, C. A. Bunton, and M. M. Mhala, *J. Am. Chem. Soc.*, **104**, 6654 (1982).
5) H. Al-Lohedan and C. A. Bunton, *J. Org. Chem.*, **47**, 1160 (1982).
6) R. A. Moss, K. W. Alwis, and G. O. Bizzigotti, *J. Am. Chem. Soc.*, **105**, 681 (1983).
7) R. A. Moss and G. O. Bizzigotti, *J. Am. Chem. Soc.*, **103**, 6512 (1981).
8) Y. Murakami, A. Nakano, and K. Akiyoshi, *Bull. Chem. Soc. Jpn.*, **55**, 3004 (1982).
9) R. A. Moss and Y. Ihara, *J. Org. Chem.*, **48**, 588 (1983).
10) Y. Okahata, R. Ando, and T. Kunitake, *J. Am. Chem. Soc.*, **99**, 3067 (1977).
11) Y. Okahata, R. Ando, and T. Kunitake, *Bull. Chem. Soc. Jpn.*, **52**, 3647 (1979).
12) F. Cramer, W. Saenger, and H. Ch. Spatz, *J. Am. Chem. Soc.*, **89**, 14 (1967).