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Pressure Effects on the Hydrolysis of 3-Nitro-4-acyloxybenzoic Acids Catalyzed by Hexadecyltrimethylammonium Bromide Micelle[1]

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The rates of the hydrolysis of 3-nitro-4-acetoxybenzoic acid (NABA), 3-nitro-4-propionyloxybenzoic acid (NPBA), 3-nitro-4-butyryloxybenzoic acid (NBBA), and 3-nitro-4-valeryloxybenzoic acid (NVBA), catalyzed by the hexadecyltrimethylammonium bromide (HTAB) micelle, were measured at pressures up to 2 kbar at 25 °C in Tris buffer solution (0.01 M,† pH 8.7). The ester binding constant K increased and the rate constant of the hydrolysis catalyzed by micelle k_{m} decreased with longer alkyl chains of esters at 1 bar. The k_{m} values were separated into the contribution of k_{OH} (hydroxide ion catalyzed hydrolysis) and k_{T} (Tris catalyzed hydrolysis), which increased monotonically up to 2 kbar. On the other hand, the K values decreased and then inversed with minima at about 1.5 kbar except for that of NABA. From the pressure dependence of log K, the volume changes, ΔV, accompanying the ester binding to the HTAB micelle at 1 bar were estimated to be in the range of 11 ± 2 cm^3/mol for NABA to 23 ± 2 cm^3/mol for NVBA. The activation volumes for the hydrolysis catalyzed by micelle, were measured as -6 ± 2 cm^3/mol for $\Delta V^{*}_{\mathrm{OH}}$ and -16 ± 2 cm^3/mol for $\Delta V^{*}_{\mathrm{T}}$ for each ester. The activation volumes for the spontaneous hydrolysis were measured as -5 ± 2 cm^3/mol for $\Delta V^{*}_{\mathrm{OH}}$ and -16 ± 2 cm^3/mol for $\Delta V^{*}_{\mathrm{T}}$ for each ester. The reaction mechanism was discussed in terms of ΔV and ΔV^{*}.

Hydrolysis of esters catalyzed by micelles as the enzyme-model is recognized to be markedly accelerated above the critical micelle concentration (cmc) and to follow the kinetics of Michaelis-Menten type.[2,3] Such an accelerating effect is explained by the incorporation of esters into the micellar phase, which is caused by the driving force of the electrostatic and hydrophobic interactions between esters and micelles. In fact, the cooperative effect of electrostatic and hydrophobic interactions on the substrate binding is important for some kinds of protease.

In the previous report,[4] we have studied about the HTAB micelle-system concerning the hydrophobic interaction as the driving force of the incorporation of neutral esters into the micellar phase. The volume change ΔV accompanying the ester binding to the micellar system at 1 bar was in the range of 0 cm^3/mol for *p*-nitrophenyl acetate (PNPA) to 18 cm^3/mol for *p*-nitrophenyl hexanoate (PNPH), which means the increment of the positive volume of 2.4 cm^3/mol for methyl or methylene group of esters for the transfer of ester molecules from an aqueous environment to the micellar phase. While, ΔV at the formation of the electrostatic interaction (charge neutralization) for 1:1 electrolytes is believed to be around 10 cm^3/mol.[5] So, we can expect that the pressure effects on the electrostatic interactions are larger than hydrophobic effects, especially for an ionic ester molecule containing only a short alkyl chain. The study about the system containing both interactions above as a driving force of the incorporation of ester into the micellar phase is meaningful to clearify the more detail mechanism of protease.

In the present work, the hydrolysis rate of anionic esters of 3-nitro-4-acyloxybenzoic acids catalyzed by a cationic HTAB micelle was measured at the pressures up to 2 kbar at 25 °C to obtain the pressure dependences of the substrate bindings and the micelle-catalyzed hydrolysis. The reaction mechanism is discussed in terms of the volume change for the formation of the micelle–ester complex and the activation volumes for the hydrolysis catalyzed by micelle, being compared with the previous date.[4]

Experimental

Materials. NABA, NPBA, NBBA, and NVBA synthesized[6] and HTAB were purified by previous method,[4] except NABA and NPBA to be recrystallized 5 times from benzene–diisopropyl ether. NABA: mp 156—158 °C (lit,[7] mp 156—158 °C); MS (75 eV), Found: *m/e* 225.02745. Calcd for $C_9H_7N_1O_6$: M, 225.02734. NPBA: mp 143.5—145.5 °C. Found: *m/e* 239.04252. Calcd for $C_{10}H_9N_1O_6$: M, 239.04300.

Apparatus and Procedure. The measurement of the hydrolysis reaction up to 2 kbar is described in previous report.[4] The rate was determined by observing the change in the optical density at $\lambda_{\mathrm{max}}=410$ nm of 3-nitro-4-hydroxybenzoate anion at pH 8.7 in 0.01 M Tris buffer solution up to 2 kbar at 25 °C±0.5 °C. The cmc of HTAB at pH 8.7 in 0.01 M Tris buffer solution up to 2 kbar was determined by the measurement of the absorption spectra of salicylic acid ($\lambda_{\mathrm{max}}=295$ nm) as a probe at 25 °C.

Results and Discussion

The apparent rate constant, k_{app}, of the hydrolysis at 1 bar increases suddenly above cmc of HTAB as shown in Fig. 1. Assuming that one ester molecule is incorporated into a micelle and that the aggregation number, N, of the micelle is independent of ester incorporation, Bunton *et al.*[8] showed that the scheme of the enzyme-catalyzed reaction in Eq. 1 is applicable to the present kinetic behavior,

$$\mathrm{M} + \mathrm{S} \overset{K}{\rightleftharpoons} \mathrm{MS} \xrightarrow{k_{\mathrm{m}}} \mathrm{M} + \mathrm{P}, \quad \mathrm{S} \xrightarrow{k_{\mathrm{s}}} \mathrm{P}. \tag{1}$$

Here M, S, MS, P, K, k_{m}, and k_{s} denote the micelle, esters the micelle–ester complex, products the binding constant between esters and micelles, the rate constants of hydrolysis catalyzed by micelle, and that of the spontaneous hydrolysis, respectively. The concentration of micelle is given by Eq. 2,

$$[\mathrm{M}] = (C_{\mathrm{D}}-\mathrm{cmc})/N. \tag{2}$$

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† 1 M=1 mol dm^{-3}.

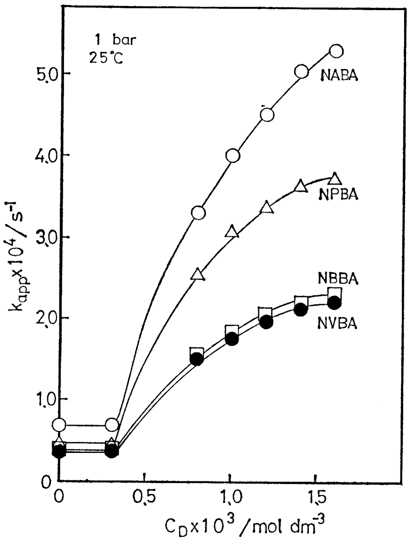


Fig. 1. Hydrolysis of anionic esters in HTAB micelle at 1 bar, pH 8.7 (0.01 M Tris buffer) and 25 °C. Each ester concn is 2.0×10^{-4} M.

Table 1. Cmc and aggregation numbers of HTAB micelle at various pressures and 25 °C

P/kbar	0.001	0.5	1.0	1.5	2.0
10^{-4}(cmc)/M	2.9	3.4	3.7	3.5	3.4
$N^{P,a)}$	95	60	52	60	76

a) Ref. 4.

Here C_D is the total concentration of HTAB. The cmc values of HTAB in our experimental conditions at each pressure are shown in Table 1 with values of the aggregation numbers, N, of HTAB micelle. The values of K and k_m determined by Lineweaver-Burk plots[4] in Fig. 2 for anionic esters at 1 bar and Fig. 3 for NBBA up to 2 kbar, are shown in Table 2 with the values of k_s at various pressure.

Ester Binding. From NMR studies[9,10] of benzoate and alkyl phenol (C_nH_{2n+1}-⌬-OH, $n=1$, 2, 3) in micellar solutions, benzoate ions exist near the micellar surface, in which the aromatic ring is located between quaternary ammonium head groups of HTAB molecules. Alkyl phenol molecules penetrate more into the micelle with increasing alkyl chains. Therefore, NABA is expected to be incorporated near the micellar surface and the other anionic esters with longer alkyl chains of acyl groups penetrate more into the hydrocarbon core of the micelle. The results mean the difference of the site of solubilization into the micelle between NABA and longer alkyl chains of esters. So that the inversion phenomena at plots of log K *vs.* pressure as shown in Fig. 4 correspond to the appearence of a maximum of the cmc of HTAB at 1 kbar in Table 1. The linear decrease of log K of NABA with increasing pressure was also observed in the systems of PNPA–HTAB[4] and methyl acetate–dodecyl hydrogensulfate micelle.[11] The change of volume ΔV being accompanied by the formation of MS complex at 1 bar in Table 3 is determined from the initial slope of plots of log K *vs.* pressure.

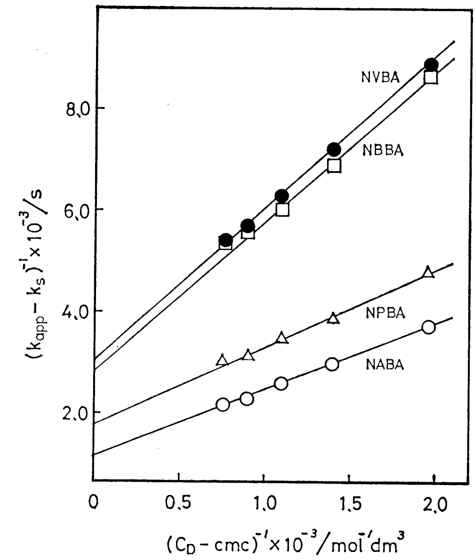


Fig. 2. Lineweaver-Burk plots for anionic esters in HTAB micelle at 1 bar, pH 8.7 (0.01 M Tris buffer) and 25 °C. Each ester concn is 2.0×10^{-4} M.

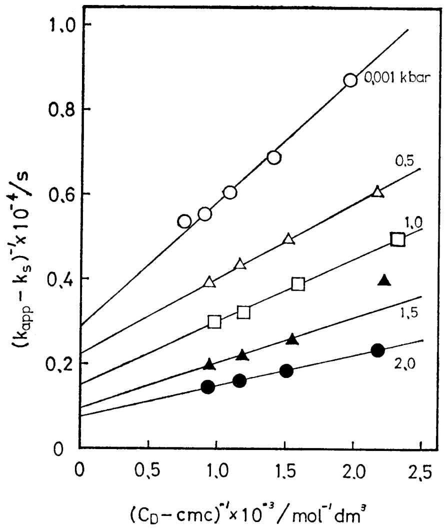


Fig. 3. Lineweaver-Burk plots for NBBA in HTAB micelle under five pressures, pH 8.7 (0.01 M Tris buffer) and 25 °C. Each ester concn is 2.0×10^{-4} M.

The K value increases with increasing alkyl chain of acyl groups of esters, and it is larger than that of neutral ester[4] at 1 bar and 25 °C except NVBA. The fact means that the electrostatic interaction in addition to the hydrophobic interaction plays an important role in the driving force of the incorporation of anionic esters with micelles. If the driving force for the ester incorporation is a sum of the electrostatic and hydrophobic interactions, the volume change ΔV is given by Eq. 3,

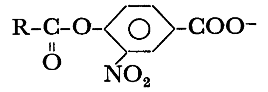
$$\Delta V = \Delta V_{\mathrm{ele}} + \Delta V_{\mathrm{H}\phi}. \qquad (3)$$

Here ΔV_{ele} and $\Delta V_{\mathrm{H}\phi}$ denote the volume changes of dehydration being accompanied by electrostatic and hydrophobic interactions between a ester molecule and a HTAB micelle, respectively. If an anionic charge of esters is neutralized completely by the electrostatic

TABLE 2. KINETIC PARAMETERS OF THE BASIC HYDROLYSIS OF ANIONIC ESTERS CATALYZED BY HTAB MICELLE AND SPONTANEOUS HYDROLYSIS AT VARIOUS PRESSURES AND 25 °C

P/kbar	$K\times10^{-4}$/M^{-1}				$k_m\times10^4$/s^{-1}				$k_s\times10^4$/s^{-1}			
	NABA	NPBA	NBBA	NVBA	NABA	NPBA	NBBA	NVBA	NABA	NPBA	NBBA	NVBA
0.001	7.34	11.3	11.7	13.5	9.83	5.95	3.48	3.13	0.647	0.457	0.390	0.367
0.5	6.11	8.42	7.88	8.10	16.9	11.2	5.00	4.95	0.931	0.670	0.584	0.538
1.0	4.28	6.98	5.37	5.36	21.2	14.4	7.65	8.96	1.52	1.12	0.997	0.900
1.5	3.88	5.63	5.34	4.78	33.9	22.7	12.5	13.8	2.25	1.67	1.53	1.36
2.0	3.09	6.26	5.43	4.87	55.1	40.4	20.2	21.6	3.39	2.57	2.41	2.09

TABLE 3. VOLUME CHANGES ACCOMPANYING THE FORMATION OF MS COMPLEX (ΔV), THE ACTIVATION VOLUMES OF THE HYDROLYSIS FOR TRIS- AND HYDROXIDE ION-CATALYZED HYDROLYSIS (ΔV^*_T AND ΔV^*_{OH}) IN MICELLAR AND NONMICELLAR SYSTEMS AT 1 bar AND 25 °C

Substrates R 	Volume changes[a)]/cm^3 mol^{-1}				
	ΔV	$(\Delta V^*_T)_{micelle}$	$(\Delta V^*_{OH})_{micelle}$	ΔV^*_T	ΔV^*_{OH}
CH_3-	11	−16	−6	−16	−5
C_2H_5-	13				
n-C_3H_7-	18				
n-C_4H_9-	23				
p-Nitrophenyl esters[b)]					
CH_3-	0	−19	−6	−18	−3
C_2H_5-	4				
n-C_3H_7-	9				
n-C_4H_9-	14				

a) ±2 cm^3/mol. b) From Ref. 4.

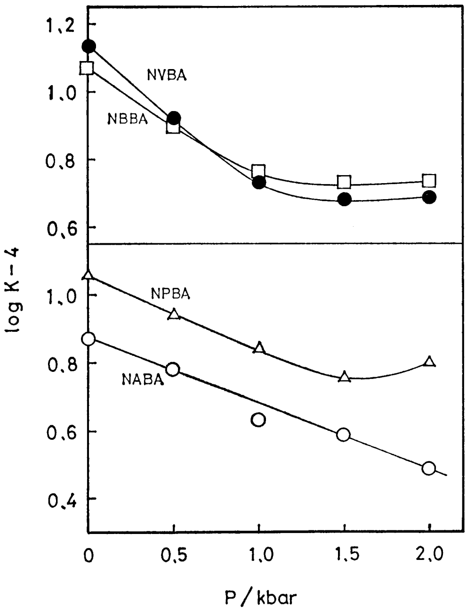


Fig. 4. Log K vs. P at 25 °C.

interaction on the transfer of esters from the aqueous environment to that of the polycationic micelle, we can estimate about 11 cm^3/mol as ΔV_{ele} for the release of the electrostriction around ionic esters as 1:1 electrolyte from the neutralization process such as $CH_3COO^- + H^+ \longrightarrow CH_3COOH$.[12)] On the base of ΔV_{ele}=11 cm^3/mol, the calculated $\Delta V_{H\phi}$ values for anionic esters are 0 cm^3/mol for NABA, 3 cm^3/mol for NPBA, 7 cm^3/mol for NBBA, and 12 cm^3/mol for NVBA. They are in good agreement with $\Delta V_{H\phi}$ values for series of neutral p-nitrophenyl esters[4)] with the hydrophobic interaction alone on the incorporation process. Therefore, ΔV values of anionic esters may be explained by the dehydration through both electrostatic and hydrophobic interactions between ester molecules and HTAB micelle.

Hydrolysis in Micelle. The rate constant k_s of spontaneous hydrolysis of anionic esters in Tris buffer solution can separate into the contributions of hydroxide ion and free-amine Tris catalysis as given by Eq. 4,

$$k_s = k_{OH}[OH] + k_T[Tris]. \quad (4)$$

Table 4 shows the k_{OH} and k_T values of NABA, determined by the same method of Neuman *et al.*[13)] In the micellar catalyst,[4)] the hydroxide ion concentration is higher near the HTAB micellar cations, while the distribution of neutral free-amine Tris would not be influenced by the micelle. Therefore, the pseudo-first-order rate constants for the hydroxide ion catalyst in micelle, $(k_{OH})_{micelle}$, are given by Eq. 5,

$$(k_{OH})_{micelle} = k_{OH}/\langle OH\rangle_{micelle}. \quad (5)$$

Here $\langle OH\rangle_{micelle}$ is the average concentration of the hydroxide ions near the micellar phase determined by using the previous date.[4)] The $(k_T)_{micelle}$ values were also determined from Eq. 6 with the known $(k_{OH})_{micelle}$ values. The values of $\Delta V_{OH}{}^*$ and $\Delta V_T{}^*$ of NABA

$$k_m = (k_{OH})_{micelle}\langle OH\rangle_{micelle} + (k_T)_{micelle}[Tris] \quad (6)$$

Table 4. Rate constants for hydrolysis of NABA at various pressures and 25 °C[a)]

P/kbar	k_{OH}/M^{-1} s^{-1},[b)]	$k_T \times 10^3$/M^{-1} s^{-1},[c)]
0.001	7.35	2.73
0.5	8.07	3.22
0.75	8.33	4.42
1.0	9.43	4.95
1.25	8.45	6.46
1.5	9.63	7.77
1.75	11.5	8.00
2.0	11.2	10.7

a) Ester concn *ca.* 5.0×10^{-5} M. b) Calcd from the Eq. $k = k_{OH}[OH] + k_T[Tris]$ using date from experiments at both 0.01 and 0.05 M total buffer concns. c) Calcd for total buffer concn.

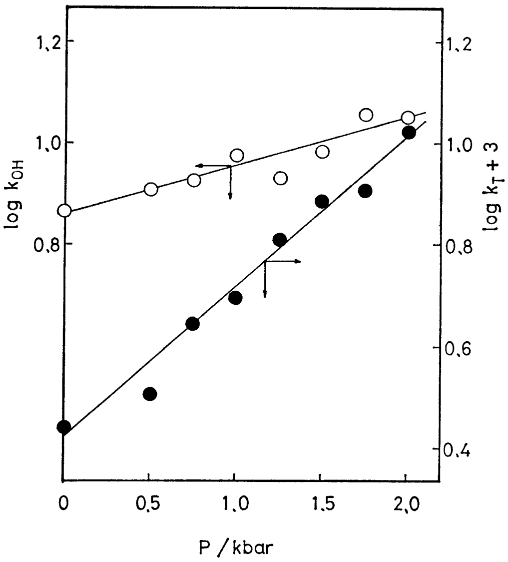


Fig. 5. Log k_{OH} and log k_T *vs.* P for NABA in nonmicellar system at 25 °C.

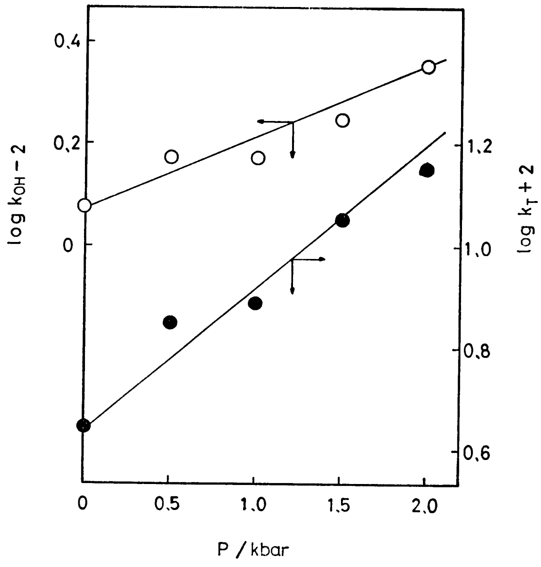


Fig. 6. Log k_{OH} and log k_T *vs.* P for NABA in micellar system at 25 °C.

for the spontaneous and micellar hydrolysis in Table 3 were obtained by the linear relation of logarithms of k_{OH} and k_T in each system (Figs. 5 and 6).

In nonmicellar systems, the value of ΔV_{OH}^* of -5 cm^3/mol for anionic esters is slightly more negative value than that of -3 cm^3/mol for nonionic esters. The ΔV_{OH}^* value should be given by a balance between the negative volume change for the bimolecular reaction and the positive volume change for the difference of the solvation effect between reactants and the activation complex. The former is expected to have a negative value of *ca.* -10 cm^3/mol for the formation of one covalent bond,[14)] for both anionic and nonionic esters. In latter case, it may be expected that there is a difference between anionic and nonionic esters in the solvation effect accompanying the formation of the tetrahedral intermediate. When a ester molecule reacts with a hydroxide ion, the charge dispersion of the activation complex takes place accompanying the formation of the tetrahedral intermediate in the transition state. The partial desolvation effect of hydroxide ions at the initial state with the formation of the tetrahedral intermediate contributions to the positive volume change. Therefore, the difference of ΔV_{OH}^* between -3 cm^3/mol for PNPA and -5 cm^3/mol for NABA is correspond to the difference of the desolvation effect on the base hydrolysis between anionic and nonionic esters.

In the micellar system, the solvation effect of water molecules both on the reactants and on the activated complex may be negligible due to the incorporation into the hydrophobic atmosphere and polyions of HTAB micelle. Therefore, the value of $(\Delta V_{OH}^*)_{micelle}$ for NABA is in good agreement with that of PNPA, which has been explained by the only contribution of the formation of one covalent bond without solvation effect.

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