Catalytic Hydrolysis of *p*-Nitrophenyl Esters in the Presence of Representative Ammonium Aggregates. Specific Activation of a Cholesteryl Nucleophile Bound to a Dialkylammonium Bilayer Membrane[1)]

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Catalytic hydrolysis of *p*-nitrophenyl esters (acetate and nonanoate) by hydroxamate and imidazole nucleophiles was studied at 30 °C in the presence of aqueous aggregates of single-chain (hexadecyl), double-chain (didodecyl and dioctadecyl) and triple-chain (trioctyl) ammonium amphiphiles. These three types of ammonium salts give rise to very different aggregate morphologies. The rate constant of ester cleavage by the nucleophiles was enhanced several hundred folds in the presence of these hydrophobic aggregates. Simple long-chain nucleophiles possessed esterolytic reactivities which reflect the hydrophobic microenvironment of the respective aggregates: single-chain<double-chain<triple-chain. On the other hand, the cholesteryl ester of imidazolecarboxylic acid showed an especially high reactivity when bound to the didodecylammonium bilayer. Cholic acid-derived nucleophiles showed normal reactivity patterns. Apparently, specific binding of the cholesterol derivative to the bilayer is responsible for the unusual catalytic behavior.

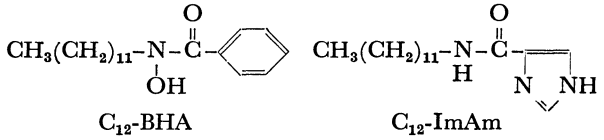
The reactivity of hydrophobic, anionic nucleophils toward *p*-nitrophenyl esters is greatly enhanced in the presence of cationic micelles of hexadecyltrimethylammonium bromide (CTAB).[2)] An additional rate-enhancing effect of *ca.* 100 fold has been observed with the aqueous aggregate of trioctylmethylammonium chloride.[3)] These results can be explained by the formation of *hydrophobic ion pairs* between anionic nucleophiles and cationic surfactants.[4,5)]

Recently we found that a series of dialkylammonium amphiphiles formed stable bilayer structures in dilute aqueous solution(10^{-2}—10^{-4} M)(1 M=1 mol dm^{-3}).[6-8)] According to the electron microscopic examination, these structures are quite similar to that of phospholipid membranes and further aggregation of the bilayer resulted in the vesicle and lamellar structures.

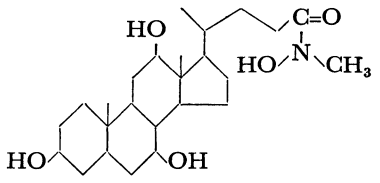
Thus, we have three distinct types of ammonium aggregates. An ammonium amphiphile which contains single, long alkyl chain (conventional surfactant) forms fluid, globular micelles at 10^{-2}—10^{-3} M. Alkylammonium salts with two long alkyl chains produce huge (10^6—10^7 daltons) aggregates with highly organized structure at 10^{-2}—10^{-4} M. An ammonium salt with three octyl chains forms fairly tight, small aggregates at 10^{-4}—10^{-5} M. Therefore, it is important to compare the influences of these ammonium aggregates on micelle-catalyzed reactions. In addition, the catalytic behavior in the ammonium bilayer would be interesting as a model of the action of membrane-bound enzymes.

In the present study, the catalytic hydrolysis of phenyl esters were examined in the presence of representative, aqueous aggregates. *p*-Nitrophenyl acetate (PNPA) and *p*-nitrophenyl nonanoate (PNPN) were used as substrate. Nucleophiles employed were *N*-dodecylbenzohydroxamic acid (C_{12}-BHA), *N*-dodecyl-4-imidazolecarboxamide (C_{12}-ImAm), *N*-methylcholohydroxamic acid (chol-HA), *N*-(4-imidazolylmethyl)-cholohydroxamic acid (chol-ImHA), *N*-[2-(4-imidazolyl)ethyl]cholamide(chol-His) and cholesteryl 4-imidazolecarboxylate(cholest-Im). The first two nucleophiles contain a long alkyl chain and the rest contain the steroidal skeleton. Their structures and abbreviations are shown below, together with those of substrates and surfactants.

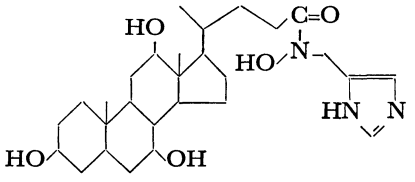
Nucleophile



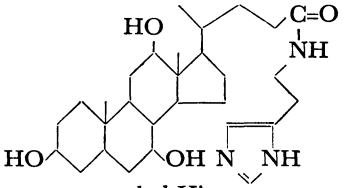
C_{12}-BHA C_{12}-ImAm



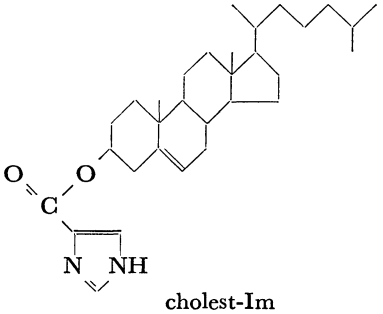
chol-HA



chol-ImHA

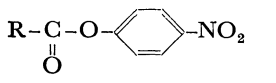


chol-His



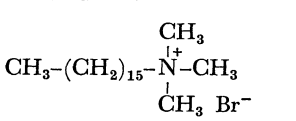
cholest-Im

Substrate

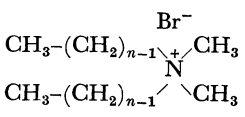


($R=CH_3$; PNPA
$R=CH_3(CH_2)_7$; PNPN)

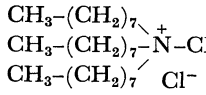
Ammonium salt



CTAB
($C_{16}N^+3C_1$)



$n=12$; $2C_{12}N^+2C_1$
$n=18$; $2C_{18}N^+2C_1$



TMAC
($3C_8N^+C_1$)

Experimental

N-Methylcholohydroxamic Acid (chol-HA). Cholic acid (4.09 g, 0.010 mol) was dissolved in a mixture of 80 ml of dry tetrahydrofuran (THF) and 20 ml of dry acetonitrile, and 1.38 g (0.012 mol) of *N*-hydroxysuccimide was added. To the resulting homogeneous solution were added dropwise 2.06 g (0.010 mol) of dicyclohexylcarbodiimide in 20 ml of dry THF at 10—15 °C. The mixture was stirred at room temperature for 2 h and the precipitates of *N,N'*-dicyclohexylurea were removed. Chloroform (300 ml) was added to the filtrate and the solution was washed with aqueous sodium carbonate (100 ml×2) and with water (100 ml×2) and dried over sodium sulfate. Chloroform was removed *in vacuo* and the white solid was recrystallized two times from a 1: 1 mixture of ethyl acetate and hexane: mp 119—120 °C, yield 2.50 g (55%). The product was confirmed by IR and NMR spectroscopies.

The succimide ester of cholic acid thus obtained (2.50 g, 0.005 mol) was dissolved in 50 ml of dry *N,N*-dimethylformamide (DMF) and added dropwise at 0 °C to 100 ml of dry DMF which contained 1.70 g (0.020 mol) of *N*-methylhydroxylamine hydrochloride and 2.0 g (0.020 mol) of triethylamine. Stirring was continued for 13 h at room temperature. The precipitates of triethylamine hydrochloride was separated and the solvent was removed *in vacuo*. The yellowish solid residue was recrystallized two times from a 1: 1 mixture of ethanol and water to give colorless needles: mp 215—216 °C, yield 1.8 g (41%). Found: C, 68.32; H, 10.04; N, 3.41%. Calcd for $C_{25}H_{43}O_5N$: C, 68.65; H, 9.84; N, 3.20%.

N-[2-(4-Imidazolyl)ethyl]cholamide (chol-His). This compound was prepared from the succimide ester of cholic acid and histamine dihydrochloride in the presence of triethylamine by a procedure similar to that mentioned above: colorless granules, yield 78%, mp 125—126 °C. Found: C, 69.49; H, 9.08; N, 8.48%. Calcd for $C_{29}H_{47}O_4N_3$: C, 69.46; H, 9.38; N, 8.38%. This procedure gave a better yield with higher purity than the previous method[9] in which methyl chloroformate was used as the condensation agent.

N-(4-Imidazolylmethyl)cholohydroxamic Acid (chol-ImHA). The succimide ester of cholic acid and *N*-(4-imidazolylmethyl)-*O*-benzylhydroxylamine dihydrochloride[10] was allowed to react similarly to give benzyl *N*-(4-imidazolylmethyl)cholohydroxamate, which was then hydrogenated over 5% Pd/$SrCO_3$ in ethanol. Colorless granules were obtained by recrystallizations (twice) from acetonitrile: mp 166—168 °C, yield 49%. Found: C, 66.52; H, 8.78; N, 8.14%. Calcd for $C_{28}H_{45}O_5N_3$: C, 66.80; H, 8.95; N, 8.35%.

Cholesteryl 4-Imidazolecarboxylate (cholest-Im). 4-Imidazolecarbonyl chloride[11] (2.8 g, 0.016 mol), 5.3 g (0.01 mol) of cholesterol and 6 g (0.06 mol) of triethylamine were added to 50 ml of chloroform and the mixture was refluxed for 4 h. Solvent was removed *in vacuo*, and the residual yellow solid was washed with a small amount of water and recrystallized three times from ethanol: pale yellow granules, mp 225—230 °C. Found: C, 76.68; H, 9.90; N, 6.00%. Calcd for $C_{31}H_{48}O_2N_2$: C, 76.86; H, 9.92; N, 6.08%.

Other Materials. *p*-Nitrophenyl acetate (PNPA) (mp 78 °C),[12] *p*-nitrophenyl nonanoate (PNPN) (bp 165 °C/0.3 mmHg (1 mmHg=133.322 Pa))[13] were prepared by the procedure reported before.

Commercial hexadecyltrimethylammonium bromide(CTAB) was recrystallized twice from water, and commercial trioctylmethylammonium chloride (TMAC) (Dojin Chemicals, Co.) was used without further purification. Didodecyldimethylammonium bromide ($2C_{12}N^+2C_1$) (Eastman Kodak) was recrystallized two times from ethyl acetate; mp 55—56 °C. Dioctadecyldimethylammonium bromide ($2C_{18}N^+2C_1$) was prepared by reaction of *N,N*-dimethyloctadecylamine and octadecyl bromide in refluxing ethanol in the presence of sodium carbonate and purified by repeated recrystallizations from ethyl acetate: colorless granules, mp 90—93 °C. Found: C, 70.04; H, 12.78; N, 2.18%. Calcd for $C_{38}H_{80}NBr\cdot H_2O$: C, 70.32; H, 12.73; N, 2.15%.

Kinetics. The hydrolysis was carried out mostly in 3 v/v % EtOH–H_2O at 30 °C, μ=0.01 (KCl), 0.01 M borate buffer (pH 7—10). The reaction rate was determined by using the absorbance of *p*-nitrophenolate at 401 nm (λ_{max}) with a Hitachi 200 UV-visible spectrophotometer. The pH value of the reaction medium varied less than 0.05 pH during the reaction (HM-10B digital pH meter, Toa Electronics).

Results

Dye Binding to Ammonium Aggregates. The absorption maximum of Methyl Orange shows hypsochromic shifts in less polar media: 465 nm in water and 420—430

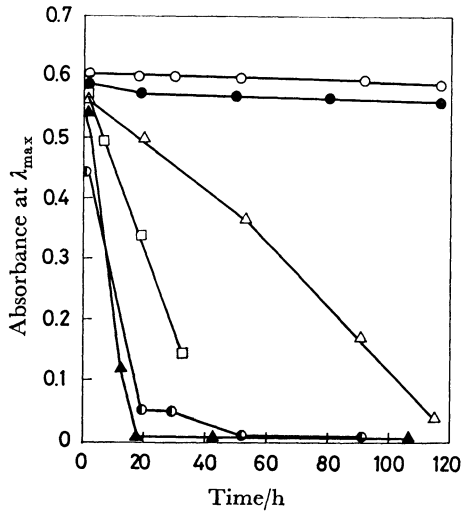


Fig. 1. Dialysis of Methyl Orange which are bound to ammonium aggregates.
pH 8.5, 0.01 M borate buffer, [Methyl Orange]=2×10^{-5} M.
▲: No ammonium salt, λ_{max}=465 nm. ○: $2C_{18}N^+2C_1$ (1×10^{-3} M), λ_{max}=410 nm. ◐: $2C_{18}N^+2C_1$(1×10^{-5} M), λ_{max}=450 nm. ●: $2C_{12}N^+2C_1$(1×10^{-3} M), λ_{max}=420 nm. △: CTAB(1×10^{-2} M), λ_{max}=430 nm. □: TMAC(2×10^{-4} M), λ_{max}=430 nm.

nm in organic media. Therefore, this compound has been used frequently for estimating the microscopic polarity of micelles[3] and polymers.[14,15] Methyl Orange (2×10^{-5} M) was dissolved in 10 ml of aqueous ammonium salts (10^{-3}—10^{-5} M) and these solutions in cellulose tubing (Visking Co.) were dialyzed against water. The residual Methyl Orange was determined at its absorption maximum.

As shown in Fig. 1, Methyl Orange is retained even after 120 h in the presence of 10^{-3} M of $2C_{12}N^+2C_1$ or $2C_{18}N^+2C_1$. Interestingly, however, Methyl Orange is rapidly lost in the presence of 1×10^{-5} M of $2C_{18}N^+2C_1$. Methyl Orange passes through the cellophane tube easily in the case of 2×10^{-4} M of TMAC, but the dialysis rate was slower in the presence of 10^{-2} M of CTAB.

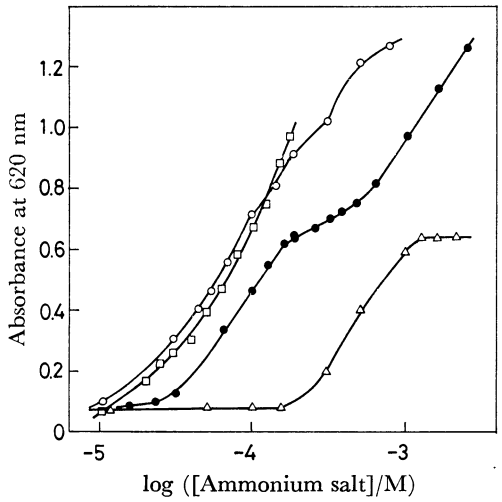


Fig. 2. Influence of hydrophobic ammonium salts on the dissociation of 2,6-dichlorophenolindophenol. 30 °C, 3 v/v % EtOH–H_2O, μ=0.01 (KCl), 1×10^{-4} M 2,6-dichlorophenolindophenol sodium salt, 3×10^{-4} M hydrochloric acid.
○: $2C_{18}N^+2C_1$. ●: $2C_{12}N^+2C_1$. □: TMAC. △: CTAB.

Dissociation of 2,6-dichlorophenolindophenol (*N*-(*p*-hydroxyphenyl)-2,6-dichloro-*p*-benzoquinone 4-imine) is often used for the measurement of the critical micelle concenctration (CMC) of cationic micelles:[3,16] λ_{max}= 500—520 nm for the undissociated species and λ_{max}= 600—650 nm for the dissociated species. Figure 2 shows the dependence of the absorbance at 620 nm, Abs_{620}, on the concentration of ammonium salts. In the case of CTAB, Abs_{620} starts to increase at *ca.* 5×10^{-4} M. The dissoication is facilitated at a lower concentration (5×10^{-5} M) of $2C_{12}N^+2C_1$. The effects of $2C_{18}N^+2C_1$ and TMAC are similar to each other, and Abs_{620} starts to increase at 1×10^{-5} M in both cases.

Alkaline(Spontaneous) Hydrolysis of p-*Nitrophenyl Esters.* The alkaline hydrolysis of *p*-nitrophenyl esters was studied at pH 10.0±0.1 in the presence of ammonium aggregates. The *p*-nitrophenol release obeyed the pseudo first order rate law for more than 90% completion. The logarithm of the pseudo first order rate constant, k_{spont}, was plotted against the logarithm of

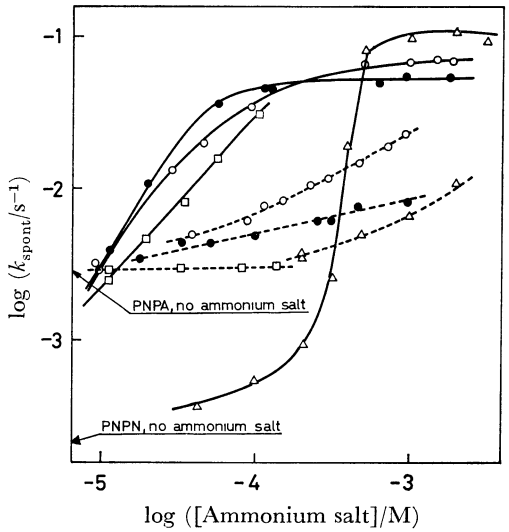


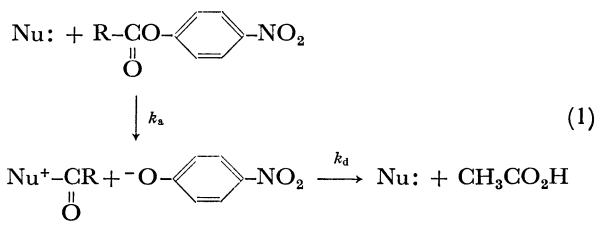
Fig. 3. Spontaneous (alkali) hydrolysis of *p*-nitrophenyl esters. 30 °C, pH 10.1±0.1, μ=0.01 (KCl), 3 v/v % EtOH–H_2O. ——: PNPN (2.00×10^{-5} M). ——:PNPA (2.00×10^{-5} M). ○: $2C_{18}N^+2C_1$. ●: $2C_{12}N^+2C_1$. △: CTAB. □: TMAC.

the ammonium concentration in Fig. 3. In the absence of ammonium salts, k_{spont} was 0.003 s^{-1} for PNPA and 0.00026 s^{-1} for PNPN. The TMAC aggregate did not affect the hydrolysis of PNPA, but micellar CTAB (1×10^{-3} M) increased the rate about two-fold. The bilayer aggregates of $2C_{12}N^+2C_1$ and $2C_{18}N^+2C_1$ accelerated the reaction by factors of 2.7 and 10 at the same concentration.

The rate enhancement was much greater with more hydrophobic PNPN substrate. k_{spont} increased by a factor of more than 100 fold upon micellization of CTAB. This must be due to efficient binding of PNPN to micellar CTAB and due to concentration of OH^- at the cationic micellar surface. The rate enhancement was observed at much lower concentrations ($<10^{-5}$ M) in the presence of other ammonium aggregates, but was saturated at 10^{-4} M. The rate saturation could not be confirmed with the TMAC aggregate because of its limited solubility.

The alkaline hydrolysis of PNPN was efficiently retarded by the increase in ionic strength: the rate decreased to 1/70 to 1/150 of the original value upon increase in ionic strength from 0.01 to 0.5 (KCl). The influence of ionic strength was larger in the bilayer systems than in the CTAB micelle.

Catalytic Hydrolysis of p-*Nitrophenyl Esters.* The catalytic hydrolysis by hydroxamate and imidazole nucleophiles proceeds *via* the acyl intermediate as in Eq. 1.



(1)

The apparent second-order rate constant of acylation is obtained by

$$k_{a,obsd} = \frac{k_{total} - k_{spont}}{[Nu]_T} \quad (2)$$

where k_{total} and k_{spont} are overall first order rate constants with and without catalyst, respectively. $[Nu]_T$ is the total concentration of nucleophile. The reaction obeyed the pseudo first order rate law for up to 90% completion. The rate constant of acyl transfer was obtained from these experiments, since excess catalyst was present in all cases relative to substrate molecules.

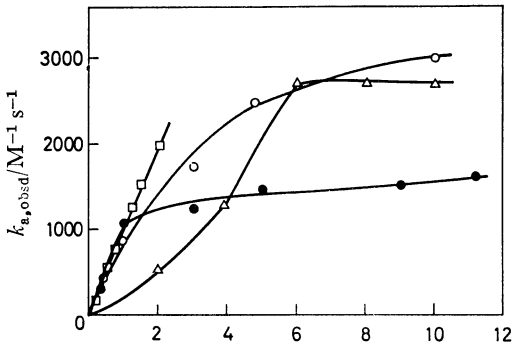


Fig. 4. Catalytic hydrolysis of PNPA by chol-HA in the presence of ammonium aggregates.
30 °C, 3 v/v % EtOH–H_2O, μ=0.01 (KCl), pH 8.9± 0.1, [PNPA]=3.79×10^{-6} M, [chol-HA]=3.02×10^{-5} M. ○: $2C_{18}N^+2C_1$. ●: $2C_{12}N^+2C_1$. △: CTAB. □: TMAC.

When chol-HA was used as nucleophilic catalyst $k_{a,obsd}$ increased in similar ways (100—300 fold) in the presence of all four ammonium aggregates as shown in Fig. 4. The rate enhanching effect is apparently related to the CMC, since efficient acceleration is noted at (2—6)$\times10^{-4}$ M for CTAB and at lower concentrations for the other ammonium salts. Similar trends were observed for other steroidal (chol-ImHA) and long-chain (C_{12}-BHA and C_{12}-ImAm) nucleophiles with rate enhancements of 100—300 fold.

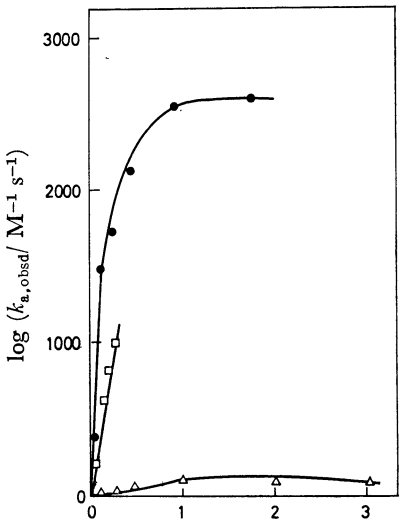


Fig. 5. Catalytic hydrolysis of PNPA by cholest-Im in the presence of ammonium aggregates.
30 °C, 3 v/v % EtOH–H_2O, μ=0.01 (KCl), pH 8.9± 0.1, [PNPA]=3.79×10^{-6} M, [cholest-Im]=3.10×10^{-5} M.
●: $2C_{12}N^+2C_1$. △: CTAB. □: TMAC

In contrast, the rate-enhancing effect for cholest-Im catalyst was more specific than those for the above-mentioned nucleophiles. As shown in Fig. 5, the nucleophilic reactivity of cholest-Im is much increased by the aggregate of TMAC and $2C_{12}N^+2C_1$, but the CTAB micelle produced a very small effect. It is interesting that cholest-Im could not be solubilized by the $2C_{18}N^+2C_1$ bilayer.

Chol-His catalyst could not be activated by any of the ammonium aggregates and $k_{a,obsd}$ was 6.5—9.2 $M^{-1}\,s^{-1}$ at pH 8.9 and 30 °C.

The $k_{a,obsd}$ values obtained for various nucleophiles in the presence of representative ammonium aggregates are collected in Table 1. The concentration of ammonium salt is 1×10^{-3} M except for TMAC in which case the concentration is 1×10^{-4} M because of its low solubility. The corresponding, less hydrophobic compounds were used to estimate $k_{a,obsd}$ in the absence

Table 1. Reaction of hydroxamate and imidazole nucleophiles with PNPA in the presence of representative ammonium aggregates[a]

Nucleophile	$k_{a,obsd}/M^{-1}\,s^{-1}$				
	None	CTAB (1×10^{-3} M)	TMAC (1×10^{-4} M)	$2C_{12}N^+2C_1$ (1×10^{-3} M)	$2C_{18}N^+2C_1$ (1×10^{-3} M)
C_{12}-BHA	15[b]	1900	6700[d]	4500	—
C_{12}-ImAm	0.09[c]	105	1200[d]	155	810
chol-HA	10	2770	2500	1600	2800
chol-ImHA	2.8[e]	113	—	320	—
chol-His	3.5	6.5	6.8	9.2	8.7
cholest-Im	0.09[c]	61	650	2570	—

a) 30 °C, pH 8.90±0.05, μ=0.01(KCl), 0.01 M borate, [PNPA]=3.79×10^{-6} M, [nucleophile]=(4.18—6.72)$\times10^{-5}$ M. b) *N*-Benzylbenzohydroxamic acid was used as nucleophile. Cited from Ref. 10. c) *N*,*N*-Dimethyl(4-imidazolecarboxamide) was used as uncleophile. Cited from Ref. 11. d) From Ref. 3. e) *N*-(4-imidazolylmethyl)benzohydroxamic acid was used as nucleophile. Cited from Ref. 10.

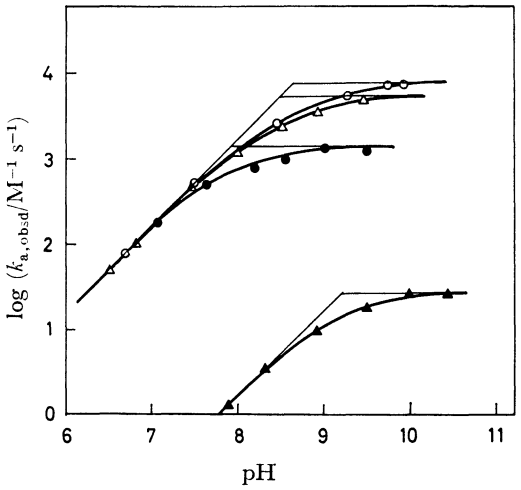


Fig. 6. pH-rate profile of the catalytic hydrolysis by chol-HA.
30 °C, 3 v/v % EtOH–H_2O, μ=0.01 (KCl), [PNPA]=3.79×10^{-6} M, [chol-HA]=3.02×10^{-5} M.
▲: No ammonium salt. △: CTAB (1.00×10^{-3} M). ○: $2C_{18}N^+2C_1$(1.00×10^{-3} M). ●: $2C_{12}N^+2C_1$(1.14×10^{-3} M).

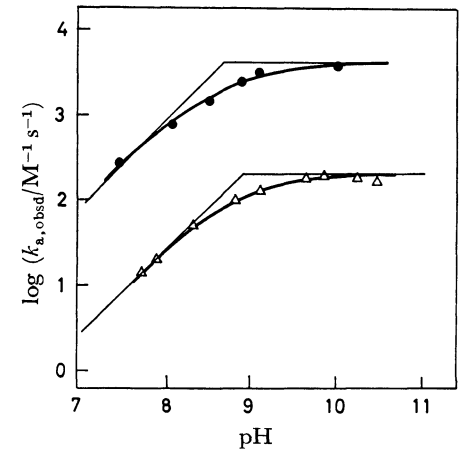


Fig. 7. pH-rate profile of the catalytic hydrolysis by cholest-Im.
30 °C, 3 v/v % EtOH–H_2O, μ=0.01 (KCl), [PNPA]=3.79×10^{-6} M, [cholest-Im]=3.10×10^{-5} M.
●: $2C_{12}N^+2C_1$ (1.14×10^{-3} M). △: CTAB (1.00×10^{-3} M).

of the ammonium aggregate when hydrophobic nucleophiles were not soluble in water.

Figures 6 and 7 are pH-rate profiles for chol-HA and cholest-Im nucleophiles, respectively, in the presence and absence of some ammonium aggregates. In all cases, log $k_{a,obsd}$ increases linearly with slope of +1 in the low pH region and plateaus are observed at the high pH. Acid dissociation constants of nucleophile, K_a, and the true second-order rate constant of the anionic species, k_a, can be estimated by curve fitting of $k_{a,obsd}$ values to the following theoretical equation.

$$k_{a,obsd} = \frac{K_a}{a_H+K_a}\cdot k_a \quad (3)$$

$$k_{a,obsd} = \alpha\cdot k_a \quad (4)$$

where a_H is the activity of hydrogen ion and α is the fraction of the dissociated nucleophile species. Solid curves in Figs. 6 and 7 are obtained by using K_a and k_a values. The agreements between the calculated curves and the corresponding experimental plots are excellent.

Table 2 summarizes pK_a and k_a values thus obtained. In the presence of the ammonium aggregate, pK_a values are lowered and k_a values are enhanced by factors of 100 to 400. The k_a value for C_{12}-ImAm is similar in the CTAB micelle and in the $2C_{12}N^+2C_1$ bilayer; however, k_a for chol-HA is larger in CTAB than in $2C_{12}N^+2C_1$ and k_a for cholest-Im is some 20 times larger in $2C_{12}N^+2C_1$ than in CTAB. These results are suggestive of the specific interaction of nucleophiles and ammonium aggregates.

Discussion

Microenvironments of Ammonium Aggregates. As briefly mentioned in the Introduction, the three types of ammonium salts form quite different aggregates. Some of their physicochemical properties are summarized in Table 3. The critical micelle (aggregate) concentrations as determined by the surface tension measurement are in the range of 5×10^{-6} to 1×10^{-3} M, and decrease approximately with increasing carbon numbers. Therefore, the critical micelle concentration is predominantly determined by the hydrophobic-hydrophilic balance of a given ammonium salt without regard to the structural specificity. On the other hand, the mass of the ammonium aggregates is varied considerably, depending on their structural characteristics.

Table 2. Acidity and rate constants for hydroxamate and imidazole nucleophiles

Ammonium salts (1×10^{-3} M)	C_{12}-BHA		C_{12}-ImAm		chol-HA		cholest-Im	
	pK_a	k_a / M^{-1} s^{-1}	pK_a	k_a / M^{-1} s^{-1}	pK_a	k_a / M^{-1} s^{-1}	pK_a	k_a / M^{-1} s^{-1}
None	9.5[b]	20[b]	13[c]	12[c]	9.2	25	13[c]	12[c]
CTAB ($C_{16}N^+3C_1$)	8.4	2560	10.3	1620	8.5	4600	8.9	194
$2C_{12}N^+2C_1$	—	—	9.7	1430	8.0	1250	8.7	3980
$2C_{18}N^+2C_1$	—	—	—	—	8.6	7000	—	—
TMAC ($3C_8N^+C_1$)[d]	—	—	9.3	5200	—	—	—	—

a) 30 °C, 3 v/v%EtOH–H_2O, μ=0.01 (KCl). b) *N*-benzylbenzohydroxamic acid was used as nucleophile. Cited from Ref. 10. c) *N*,*N*-dimethyl(4-imidazolecarboxamide) was used as nucleophile. Cited from Ref. 11. d) From Ref. 3.

Table 3. Physicochemical properties of ammonium aggregates

Ammonium salts	Total carbon number	Aggregate morphology	10^4 CMC / M	10^{-4} Aggregate weight / dalton	Methyl Orange λ_{max}/nm
Single-chain					
CTAB ($C_{16}N^+3C_1Br^-$)	19	globular micelle	8	4	430(1×10^{-2} M)[a]
Double-chain					
$2C_{12}N^+2C_1Br^-$	26	bilayer	0.5[b]	100[d]	420(1×10^{-3} M)
$2C_{18}N^+2C_1Br^-$	38	bilayer	0.05[c]	1000[d]	410(1×10^{-3} M) 450(1×10^{-5} M)
Triple-chain					
TMAC($3C_8N^+C_1Cl^-$)	25	small, tight aggregate	*ca.* 0.3	<1	430(2×10^{-4} M)

a) λ_{max} is estimated at the ammonium concentration given in the parentheses. b) A. W. Ralston, D. N. Eggenberger, and P. L. DuBrow, *J. Am. Chem. Soc.*, **70**, 977 (1948). c) H. Kunieda and K. Shinoda, *J. Phys. Chem.*, **82**, 1710 (1978). The value for the chloride salt. d) Determined by the light scattering method: Union Giken Co., Ltd., Model LS-600.

The aggregate weight of TMAC is estimated to be less than 10^4 daltons and that of CTAB is 4×10^4 daltons. In contrast, the double-chain ammonium salts form huge aggregates of 1—10 million daltons. The hydrophobic nature of these aggregates can be estimated from λ_{max} of Methyl Orange. The λ_{max} values given in Table 1 correlate roughly with the total carbon numbers. However, λ_{max} was closer to that in water when 1×10^{-5} M of $2C_{18}N^+2C_1$ was present, probably due to incomplete formation of the stable aggregate.

The facilitated dissociation of 2,6-dichlorophenolindophenol appears to reflect the critical micelle concentration. Thus, Abs_{620} abruptly increased at *ca.* $(4—8)\times10^{-4}$ M of CTAB, but it increased at ammonium concentrations as low as 10^{-5} M in the case of other ammonium salts.

The dialysis experiments indicate that Methyl Orange is retained in the dialkylammonium bilayers most effectively as shown in Fig. 1. The CTAB micelle is much less effective and the TMAC micelle lacks in the retention capability almost completely. These trends can be correlated with the aggregate weight. Undoubtedly, the dialkylammonium aggregates can retain Methyl Orange very well because of their highly-organized, stable structures. This capacity is lost at concentrations close to the CMC, as shown by the data obtained in the presence of 1×10^{-5} M of $2C_{18}N^+2C_1$.

Specific Activation by Ammonium Bilayers. The spontaneous(alkali) hydrolysis of phenyl esters is accelerated in the presence of cationic micelles. This is usually explained by assuming that hydroxide ions concentrated in the Stern layer act on the phenyl ester bound to the micellar phase.[17] The results of Fig. 3 endorse this explanation. It is clear that PNPA is weakly bound to the ammonium aggregate in contrast to the tight binding for more hydrophobic PNPN, since acceleration of the spontaneous hydrolysis is 200—300 times for PNPN but is at most 10 times for PNPA.

As shown in Table 1, the catalytic hydrolysis of PNPA is remarkably accelerated in the presence of all the ammonium aggregates: 100—1000 times rate enhancements. The pK_a values of the hydroxamate and imidazole nucleophiles are lowered by 0.3—3 pK units when they are bound to any of the aggregates of the single-chain, double-chain and triple-chain ammonium salts. At the same time, their k_a values are enhanced by factors of several hundred (Table 2). These results violate the Brönsted relationship and is explained by the concept of "*hydrophobic ion pair.*"[4,5,18]

According to this concept, prominent activation of some anionic nucleophiles is attributed to the formation of ion pairs between anionic nucleophiles and ammonium surfactants in the hydrophobic microenvironment, which usually results in pK_a lowering of the conjugate acid of the nucleophile (increase in the amount of the effective nucleophile) and the increased reactivity of the anionic nucleophile (k_a increase). The data of Table 2 clearly show that large enhancements of $k_{a,obsd}$ (Table 1) are derived from pK_a lowering and the increase in k_a. Chol-His nucleophile is not activated because it is not an anionic nucleophile under the reaction conditions used.

The extent of rate acceleration for long-chain hydroxamate and imidazole nucleophiles (C_{12}-BHA and C_{12}-ImAm) of Table 1 appears to be in general agreement with the trend of facilitated dissociation of 2,6-dichlorophenolindophenol: TMAC>$2C_{18}N^+2C_1$>$2C_{12}N^+2C_1$>CTAB. The rate-enhancing effect of the ammonium aggregate is somewhat different in the case of chol-HA, and the CTAB micelle is more effective than the $2C_{12}N^+2C_1$ bilayer. On the other hand, cholest-Im is *ca.* 40 time more activated in the $2C_{12}N^+2C_1$ bilayer than in the CTAB micelle. Furthermore, the bilayer-bound cholest-Im is more reactive than that bound to the TMAC aggregate. The reverse is true for other nucleophiles. These results cannot be explained by the hydrophobicity of the aggregate alone.

Aqueous solutions of the dialkylammonium aggregate can solubilize cholesterol, and the bilayer structure is retained in electron micrographs upon addition of one third molar cholesterol.[7] It is well known that cholesterol is an important ingredient of plasma membranes and affect the membrane fluidity.[19] Therefore, cholest-Im is conceivably bound to the ammonium bilayer in a specific manner. The specific nature of cholesterol binding may be related to the inability of the $2C_{18}N^+2C_1$ bilayer to solubilize cholesterol. On the other hand, cholic acid acts as a surfactant that disintegrates the phospholipid bilayer.[20] Similarly, the lamella structure

of the $2C_{18}N^{+}2C_1$ aggregate is destroyed when one third molar cholic acid is added.[21] Then, nucleophiles derived from cholic acid must be bound to the ammonium bilayer non-specifically.

The rate-enhancing effect of CTAB and TMAC aggregates appears to be determined predominantly by the hydrophobicity of nucleophiles and ammonium aggregates. Specific binding cannot be expected because of the fluid nature of these aggregates. In contrast, dialkylammonium salts form the stable bilayer structure similar to the phospholipid bilayer, and specific binding is quite conceivable. Why the conceivably specific binding of a cholesteric nucleophile produces better rate enhancement is not yet clear.

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