Hydrolytic Reactions of *p*-Nitrophenyl Esters in Reversed Micellar Systems

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Hydrolytic reactions of several *p*-nitrophenyl esters were studied with and without catalysts in reversed micellar systems consisting of sodium octanoate, 1-hexanol and water. Hydrolytic rate constants become very large as compared with those in aqueous solutions, increasing with decrease in the molar ratio of water to sodium octanoate (*R*-value). The results of kinetic measurements were discussed in terms of the behavior of water molecules and polar groups of sodium octanoate as revealed by means of IR and ^{1}H, ^{13}C and ^{23}Na-NMR spectroscopy. It was concluded that the decrease in the *R*-value induces the increase in the mobility of water molecules and the decrease in the polarity of the water core, which affects the partition coefficient of *p*-nitrophenyl esters between the 1-hexanol phase and the water phase, enhancing hydrolytic reactions.

A number of works[1] have been published concerning the enhancement of reaction rates at the critical micellar concentration in normal micellar systems. Attention has also been paid to reversed micellar systems, in which several reactions are much accelerated.[2-4] Fendler *et al.*[5] who extensively examined catalytic reactions in reversed micelles, pointed out that water molecules solubilized in polar cavities of reversed micellar systems are less polar than those in the bulk phase, and suggested that this is closely related to the catalytic effect. Thus, it is of interest to investigate the behavior of molecules involved in reversed micellar systems, especially the behavior of water molecules and sodium octanoate at the boundary phase between the water core and the 1-hexanol phase. This does not seem to have been fully elucidated in relation to the acceleration of chemical reactions in the systems.

We have kinetically investigated hydrolytic reactions of *p*-nitrophenyl esters in reversed micellar systems consisting of sodium octanoate, 1-hexanol and water, and also measured the catalytic activity of L-histidine and imidazole solubilized in the water core in the same system for hydrolysis of *p*-nitrophenyl acetate. The behavior of water molecules in reversed micelles was studied by means of IR and ^{1}H-NMR spectroscopy, and that of the polar group of sodium octanoate, *i.e.*, sodium ions and carbonyl groups, by ^{23}Na and ^{13}C-NMR spectroscopy. Except for the movement of the methylene group in the case of Aerosol OT in chloroform,[6] the behavior of polar groups of surfactants has scarcely been studied. The results obtained in measurements of reaction rates were discussed in terms of the characteristic behavior of molecules, ions and polar groups present at the boundary phase between the water core and the 1-hexanol phase.

Experimental

Materials. Sodium octanoate, 1-hexanol, *p*-nitrophenyl acetate (PNPA), L-histidine and imidazole (analytical grade) were used without further purification. *p*-Nitrophenyl octanoate (PNPO) and L-alanine *p*-nitrophenyl ester hydrochloride (APNP) were prepared by the condensation reaction between the corresponding acid and *p*-nitrophenol in tetrahydrofuran. Water was distilled and buffered with 0.03 M phosphate aqueous solution.

Measurements of Reaction Rates. PNPA, PNPO, and APNP were used as substrate. The initial concentration of these esters in 1-hexanol was 5×10^{-4} M. Reversed micelles were prepared by mixing three components, water (buffer solutions), 1-hexanol and sodium octanoate, and allowing them to stand at 30 °C for 24 h. 1-Hexanol solution of *p*-nitrophenyl ester was added to reversed micellar solutions, and the hydrolytic reaction rates of esters with and without catalyst were observed by measuring the absorption (OD_t) of *p*-nitrophenolate ions (400 nm) at 30 °C as a function of time with a Hitachi EPS-3T spectrophotometer. When the reaction was complete, the absorption (OD_∞) was determined from the absorption *vs.* time curve after a sufficiently long time. The results were analyzed as first order kinetics by plotting ($OD_\infty - OD_t$) in a logarithmic scale against time *t*. The slope of the line afforded the rate constant *K*. When L-histidine or imidazole was used as a catalyst, the difference in rate constants between the catalytic and noncatalytic reactions both in reversed micelles divided by the concentration of the catalyst was taken as K_c.

Spectroscopic Measurements. Infrared spectra were obtained with a Hitachi EPS-3G infrared spectrophotometer. The liquid cell used was equipped with CaF_2 windows, the optical path length being 0.05 mm. ^{1}H, ^{13}C, and ^{23}Na-NMR spectra were measured with a JEOL JNM-PS FT-NMR spectrometer operating at 100, 25 and 24 MHz, respectively. Measurement conditions for ^{13}C and ^{23}Na-NMR: spectral width 6250 and 1000 Hz, repetition 10 and 2.5 s, data point 8192 and 4096, numbers of scans 256 and 200, respectively. UV spectra of pyridine 1-oxide were observed with a Hitachi spectrophotometer model EPS-3T using a thermostated cell.

Results and Discussion

Hydrolytic Reactions of p-*Nitrophenyl Esters in Reversed Micelles.* Rate constants of hydrolysis of PNPA together with weight ratios of three components of reversed micellar systems and pH values of the phosphate buffer solution solubilized into the systems are given in Table 1. The results indicate that the rate constant increases with increase in pH. This tendency in reversed micellar systems is analogous to that in aqueous solutions.

Rate constants of hydrolysis of PNPA, PNPO, and APNP in reversed micelles are given in Table 2. When the buffer solution of the same pH is used, the hydrolytic reaction of PNPA is much more accelerated in reversed micelles as compared with that in aqueous

Table 1. Rate constants and weight ratios of the three components of reversed micelles

Water	1-Hexanol	Na Oct.	pH[a]	$K\times10^3$ (min^{-1})
2.5	6.5	1	7.7	10.3
2.5	6.5	1	7.9	14.0
2.5	6.5	1	8.0	14.5
3.0	6.0	1	7.7	9.7
3.0	6.0	1	7.9	13.0
3.0	6.0	1	8.0	13.6

a) Values of the phosphate buffer solutions solubilized into reversed micellar systems.

Table 2. Rate constants for *p*-nitrophenyl acetate (PNPA), *p*-nitrophenyl octanoate (PNPO), and L-alanine *p*-nitrophenyl ester hydrochloride (APNP) in aqueous solutions and reversed micelles

	PNPA[a]	PNPA[b]	PNPO[b]	APNP[b]	APNP[a]
$K\times10^3$ (min^{-1})	1.38	18.7	0.1	800	2.4
pH	7.9	7.9	7.9	6.5	6.5

a) In aqueous solutions. b) In reversed micelles. Concentrations of water and sodium octanoate are 12.9 and 1.38 M, respectively, in 1-hexanol ($R=9.2$).

Table 3. The catalytic rate constant of L-histidine in hydrolysis of *p*-nitrophenyl acetate in reversed micelles

R-value	$K\times10^3$ (min^{-1})	[L-his]$\times10^4$ (M)[a]	K_c ($min^{-1}M^{-1}$)
9.2	18.7	5.0	7.5
		20.0	7.3
13.9	16.7	5.0	6.5
18.4	13.8	5.0	3.3
		10.0	2.9

a) The concentration of L-histidine in the water core of reversed micelles. Concentrations of water and Na Oct are, respectively, 12.9 and 1.38 M in 1-hexanol for $R=9.2$, 12.9 and 0.93 M for $R=13.9$, and 12.9 and 0.69 M for $R=18.4$.

solutions. The hydrolytic reaction of APNP is remarkably accelerated, while that of PNPO is very slow. The order of rate constants for three *p*-nitrophenyl esters in the same reversed micellar systems (Table 2) is in line with the order of the solubility of these esters in water. The partition of the substrate between the water core and the 1-hexanol phase plays an important role in the acceleration of reaction. Ionic interaction between negative charges of sodium octanoate (carboxylate anion) and positive charges of L-alanine *p*-nitrophenyl ester hydrochloride (ammonium cation) is also an important factor in the prominent acceleration observed in the case of APNP. Judging from the fact that PNPO is hydrolyzed a little in spite of its very low solubility in water, the reaction is considered to take place at the boundary phase between the water core and the 1-hexanol phase.

In order to know how the structure of the water core and its surroundings affects the hydrolytic reaction, the dependency of the rate constant of hydrolysis of PNPA on the molar ratio of water to sodium octanoate, $R=[H_2O]/[Na\ Oct]$, was studied (Fig. 1). The rate constant increases linearly with decrease in *R*-value. An increase in the amount of sodium octanoate in the system seems to increase the amount of PNPA partitioned into the water core and its surrounding layer of the surfactant, accelerating the hydrolytic reaction. Reduction of the molar ratio of water to sodium octanoate at the same concentration of water decreases the diameter of the water core in reversed micelles, increasing the total surface area of the core in the system. The increase in the surface area of the water core is considered to raise the hydrolytic reaction rate.

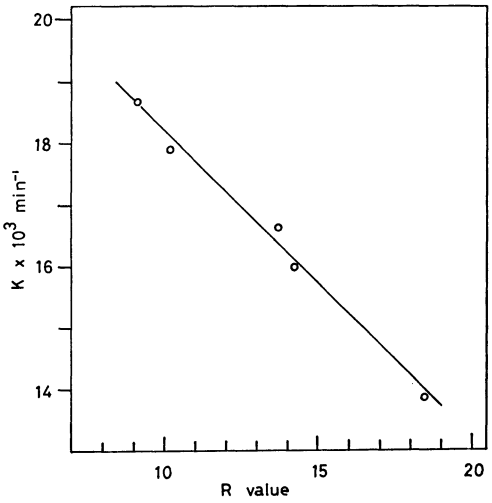

Fig. 1. Rate constant of hydrolysis of PNPA as a function of *R*-value ($[H_2O]/[Na\ Oct]$). In all cases, water concentration is 12.9 M in 1-hexanol.

Catalytic activities of L-histidine and imidazole added as a catalyst into the water core of reversed micelles were measured only in hydrolysis of PNPA. The results obtained for L-histidine are given in Table 3. The catalytic rate constant, K_c, increases with decrease in *R*-value as in the case of hydrolysis without catalyst, giving a nearly constant value in the L-histidine concentration range 5×10^{-4}—20×10^{-4} M. In the case of $R=9.2$, the catalytic rate constant is 7.5 $min^{-1}M^{-1}$, larger than the corresponding rate constant (5.7 $min^{-1}M^{-1}$) in aqueous solutions of the same pH values. In the case of $R=18.4$, however, the value of K_c (3.3 $min^{-1}M^{-1}$) is smaller than the catalytic rate constant (5.7 $min^{-1}M^{-1}$) in aqueous solutions. Thus, the probability of the presence of L-histidine at the boundary phase between the water core and the 1-hexanol phase in the above two cases would differ. Imidazole, a very active catalyst to hydrolyze PNPA in aqueous solutions, shows no catalytic activity in hydrolysis of PNPA in reversed micelles. The results reveal that the catalytic activity is dependent on the probability of the presence of the catalyst at the boundary phase in reversed micellar systems. In order to study the distribution of catalysts between 1-hexanol and water, partition coefficients, $K(C_{1\text{-hex}}/C_{aqu})$, of imidazole and L-histidine were measured and found to be 0.92 and 0, respectively. The results indicate

Table 4. Chemical shifts of protons of 1-hexanol and H_2O, and line width of H_2O peak

R-value	Chemical shift (δ)			Line width of H_2O (Hz)
	CH_3-	-CH_2-OH	H_2O	
18.4	1.24	3.90	5.18	6.61
13.6	1.24	3.90	5.20	6.45
11.6	1.25	3.90	5.24	6.31
10.3	1.27	3.90	5.24	6.01
9.2[a)]	1.28	3.90	5.26	4.95
9.2[b)]	1.28	3.90	5.27	4.95

a) Concentrations of water and sodium octanoate are 12.9 and 1.38 M, respectively. b) Concentrations of water and sodium octanoate are 14.9 and 1.61 M, respectively.

Table 5. Relative intensities of IR bands of HOD at the three wavelengths

R-value	Wavelength (cm^{-1}) of HOD band		
	3400	3550	3800
18.4	0.87	1.0	0.06
11.6	0.88	1.0	0.07
9.2	0.98	1.0	0.10

The intensity of the bond at 3550 cm^{-1} was taken as a standard.

that L-histidine molecules are concentrated at the boundary phase between the water core and the 1-hexanol phase, but not imidazole molecules due to their higher solubility into 1-hexanol. The ionic force between negative charges at the boundary phase (carboxylate anions of sodium octanoate) and positive charges of L-histidine molecules (ammonium cations) increase the probability of the presence of L-histidine at the boundary phase between the water core and the 1-hexanol phase. Thus, L-histidine molecules are concentrated at the boundary phase of reversed micelles due to their solubility and ionic force, so that L-histidine shows catalytic activity, depending on its concentration relative to that of the surfactant. This would not apply to the case of imidazole since it has higher solubility into 1-hexanol, but no positive charges.

The Structure and Behavior of Water Molecules and Polar Groups of Sodium Octanoate. In order to understand the role of the water molecule in reversed micelles in the hydrolytic reaction, their mobility and chemical environment were studied by means of ^{1}H-NMR for different values of *R*. The results are given in Table 4. The resonance peak of the water proton moves to a lower field, the line width of the water proton peak decreasing with decrease in *R*-value. For a given *R*-value, however, the line width does not change with the change in the molar ratio of water to 1-hexanol. The chemical shifts of C-1 methylene protons (–CH_2–OH) and methyl protons of 1-hexanol do not vary appreciably with *R*-value. This indicates possible structural changes in water cores of reversed micelles with change in *R*-value. The decrease in the line width of the water proton peak, *i.e.*, the increase in the mobility of water molecules in reversed micelles

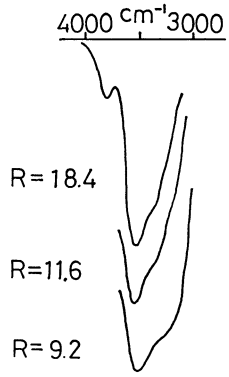

Fig. 2. Infrared spectra of HOD in reversed micelles. These spectra were taken as difference spectra between octanoic acid in 1-hexanol (reference cell) and reversed micellar solutions (sample cell).

with lowering in *R*-value seems unreasonable, since the size of the water core is considered to become smaller and consequently the relative amount of free water molecules seems to decrease with the decrease in *R*-value. For the sake of clarification, infrared spectrum due to the water molecule was taken. All the measurements were made on the uncoupled OH stretching vibration of HOD by using the solution of 5.5 M in D_2O.[7] The advantage of this approach lies in the fact that each species of water molecules gives a single, nearly gaussian absorption band. The results are shown in Fig. 2. From the absorption band due to the water molecule dispersed in pure 1-hexanol, the band at 3800 cm^{-1} is assigned to the water molecule dispersed in 1-hexanol. The band at 3400 cm^{-1} is assigned to the usual mobile (free) water by comparing the IR spectrum of pure water. Thus, the absorption band observed at 3550 cm^{-1} is due to the water molecule bound to the polar head group of the surfactant. The relative intensities of three bands, intensity of the band at 3550 cm^{-1} being taken as the standard, are given in Table 5. The relative intensity of the band at 3400 cm^{-1}, which is a measure of the mobile water molecule, increases with decrease in *R*-value. This is in line with the fact that the line width of the water proton peak decreases with decrease in *R*-value, as obtained by ^{1}H-NMR measurements.

The resonance peak of the water proton moves to a lower field with decrease in *R*-value, indicates that the property of the water molecule changes with *R*-value. Pyridine 1-oxide is a water soluble compound, the absorption maxima of which are correlated with *Z* values in several solvents.[8] The polarity of the water core of reversed micelles, measured by the absorption band shift of pyridine 1-oxide solubilized into reversed micelles (Fig. 3), depends on the ratio of water to sodium octanoate, *i.e.*, the polarity of the water core decreases with decrease in *R*-value.

In order to study the behavior of the entities at the boundary phase between the water core and the 1-hexanol phase, the mobility of the carbonyl carbon and the sodium ion of sodium octanoate was examined by means of ^{13}C and ^{23}Na-NMR, respectively. Figure 4 shows the ^{23}Na-NMR spectrum of sodium

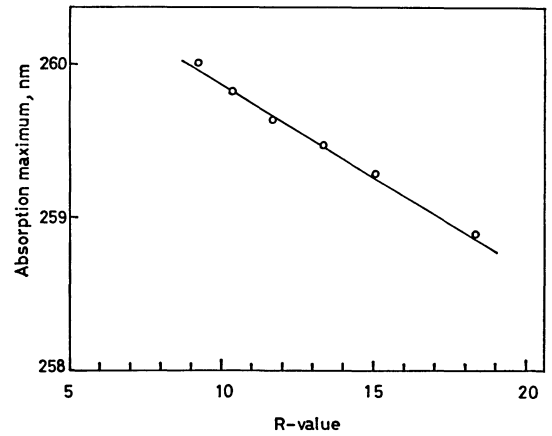

Fig. 3. Plots of absorption maxima due to pyridine 1-oxide against *R*-value. The absorption maxima due to pyridine 1-oxide in water, methanol and ethanol are 255.4, 263.3 and 265.1 nm, respectively.

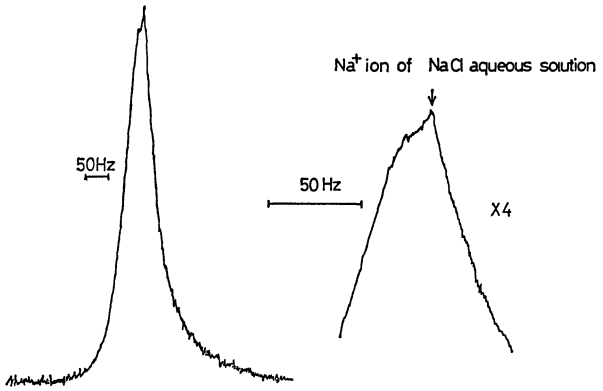

Fig. 4. ^{23}Na-NMR spectra of sodium octanoate in reversed micellar systems.

octanoate in reversed micellar system. The aqueous solution of sodium chloride was taken as an external standard, and the chemical shift of the peak due to the sodium ions in reversed micelles from the standard peak was evaluated. By using the pulse and Fourier transform technique, it has become possible to separate the resonance peak of the sodium ion in reversed micelles from that of the external standard. The chemical shifts and the line widths of the resonance peaks due to the carbonyl carbon and the sodium ion of sodium octanoate as a function of *R*-value are summarized in Table 6. When *R* is large (for instance $R=18.4$), the line width of the water proton peak in the ^{1}H-NMR spectrum (Table 4) reveals that the bound water molecules are rich. The line width and the chemical shift of the sodium ion in the ^{23}Na-NMR spectrum indicate that the mobility of the sodium ion in reversed micelles is high and the physicochemical environment of the sodium ion resembles that of a sodium ion in aqueous solutions (*i.e.*, the sodium ion is fully dissociated). When *R* is small (for instance $R=9.2$), water molecules are rather mobile, and the mobility of the sodium ion is low, so that the physicochemical environment of the sodium ion is different from that in aqueous solutions. Thus the increase in the mobility of water molecules with

Table 6. Chemical shifts and line widths of the carbonyl carbon and sodium of sodium octanoate

R-value	^{13}C-NMR[a]		^{23}Na-NMR[b]	
	Chemical shift (ppm)	Line width (Hz)	Chemical shift (ppm)	Line width (Hz)
18.4	183.4	38.4	2.08	5.4
11.6	183.1	39.7	2.79	7.3
9.2	182.6	42.7	2.97	9.8

a) Expressed in ppm using TMS as an external standard. b) Expressed in ppm using an aqueous solution of sodium chloride as an external standard.

decreasing *R*-value results from the decrease in the amount of hydrated sodium ions in the water core. The degree of dissociation of sodium ions of sodium octanoate becomes higher as *R*-value increases, and the amount of the water molecule hydrated by the sodium ion released from sodium octanoate increases with increase in *R*-value. Such a situation is also reflected in the results of ^{13}C-NMR spectra. The line width of the resonance peak of the carbonyl carbon of the surfactant decreases and the peak position is shifted to a lower field as *R* increases. The increase in the mobility and the down field shift of the carbonyl carbon signal are caused by the sodium ion released from sodium octanoate as *R* increases. Namely, the carbonyl group of the octanoate anion can move more freely and the electron density of the carbonyl group of the octanoate anion decreases by releasing the sodium ion from sodium octanoate.

In aqueous micellar systems[9] and surfactant aggregate systems in organic solvents[10] the exchange between monomers and surfactant micelles is thought to be relatively fast. However, the exchange in reversed micellar systems containing water molecules has not been clarified. The aggregation number in this system has not been measured, but from the results shown above and the fact that sodium octanoate is hardly soluble in 1-hexanol, the equilibrium constant *K* ($K=[S_m]/[S]^m$, where S_m, S, and m are the concentration of micelles, the concentration of surfactants and the aggregation number, respectively) seems to be relatively large.

Relationship between Hydrolytic Activity and the Structure of Reversed Micelles. Acceleration of the hydrolytic reaction caused by decrease in *R* may be explained as follows. The substrate becomes more soluble when the mobility of water molecules increases and the polarity of water cores decreases. The amount of substrate transferred from the 1-hexanol phase to the water core then increases. Hence, the hydrolysis is enhanced. The results of ^{23}Na and ^{13}C-NMR indicate that polar head groups of surfactants aggregate more tightly for a smaller *R*-value. Thus, the water core seems smaller for a smaller *R*-value, but this has not been confirmed by light scattering. The decrease in the diameter of the water core in reversed micelles gives rise to an increase in the specific surface area of reversed micelles, enhancing the rate of hydrolytic reaction. It was found that L-histidine is active, but not imidazole as a catalyst of hydrolysis of PNPA.

L-Histidine molecules are considered to be located at the boundary phase between the water core and the 1-hexanol phase through the ionic force between positive charges of the ammonium ion of L-histidine and negative charges of sodium octanoate and its low solubility in 1-hexanol. However, this does not apply to imidazole, since it has high solubility in 1-hexanol but no positive charge. Thus, L-histidine molecules can act as a catalyst of hydrolysis at the boundary phase of reversed micelles, but not imidazole molecules transferred from the water core to 1-hexanol phase. Hydrolytic reaction is remarkably enhanced when APNP is used as a substrate. The concentration of APNP at the boundary phase due to the ionic interaction and its high solubility in the water core may enhance the hydrolytic reaction. It is important for the reaction in reversed micellar systems to concentrate the catalyst or substrates at the boundary phase between the water core and the 1-hexanol phase.

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