Nonenzymatic Hydrolysis of Adenosine 5′-Triphosphate in Micellar and Reversed Micellar Systems

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The nonenzymatic hydrolysis of adenosine 5′-triphosphate (ATP) in micellar and reversed micellar system was examined as an approach to elucidate the function of ATPase. In aqueous micellar systems an anionic surfactant does not affect the hydrolysis rate, but a cationic surfactant, cetyltrimethylammonium bromide(CTAB), retards the hydrolysis. This deceleration effect is interpreted to be owing to the pH change or accumulation of hydroxide ions at the cationic micellar-water interface which serves as reaction site. In the reversed micellar system where aqueous ATP phase is solubilized in *n*-hexane by dodecylammonium propionate(DAP), the hydrolysis rate of ATP is enhanced by several times as compared with that in an aqueous system. Addition of Mg^{2+} or Ca^{2+} enhances the hydrolysis rate markedly only in the DAP reversed micellar system. The catalytic effect of the DAP reversed micellar system is discussed with respect to the polarity of water phase solubilized in the core of reversed micelles.

Adenosine 5′-triphosphate(ATP) plays an important role in repository and transfer of chemical energy in many enzymatic processes in living systems. Energy transfer in these systems usually involves the reactions of ATP into adenosine 5′-diphosphate (ADP). Though many studies have been made as to the structure and function of ATPase, the mechanism of its catalytic action is not yet clear. To improve this situation, some nonenzymatic model reactions have also been examined, and it has been observed that the hydrolysis reaction of ATP is enhanced either by the addition of metal ions or polyamines,[1,2] or in the presence of aprotic polar solvent.[3]

On the other hand, surfactant micelles affect rates of various reactions and are noticed as a model for enzymatic action. Recently, Fendler *et al* observed that reversed micelles in nonpolar solvents showed a significant rate enhancement in several reactions and pointed out that reversed micelles could provide a favorable model for the micro-environment of enzyme active sites.[4]

In the present investigation, we carried out the nonenzymatic hydrolysis of ATP both in an aqueous micellar system and in a reversed micellar system, and examined the micellar effect on hydrolysis rate to shed light on the function of ATPase.

Experimental

Materials. Disodium salt of ATP was purchased from Sigma Chemical Co. and was used without further purification. Cetyltrimethylammonium bromide(CTAB) and sodium dodecylsulfate(SDS) were recrystallized three times from methanol–ether. Dodecylammonium propionate(DAP) was prepared by Kitahara's method and purified three times by recrystallization from *n*-hexane;[5] mp 55—56 °C (lit, 54—56 °C)[5]. Found (Calcd): C, 69.68(69.44), H, 12.38 (12.82), N, 5.32(5.40)%. Magnesium chloride and calcium chloride of analytical grade were used after dried.

Reactions. *Reaction in Aqueous Micellar System*: A sample solution, pH of which was adjusted by using a buffer solution or addition of small amount of a *dil* NaOH solution, was prepared by dissolving ATP and surfactant *etc.* The solution was maintained at 50 °C with stirring. A pH meter of Toa HM-5A was used for pH measurement. The mixture was heterogeneous at low CTAB concentration (below about 6×10^{-3} M when ATP concentration was 2×10^{-3} M at 50 °C) because of the precipitation of CTAB–ATP salt, but at higher CTAB concentration the precipitate was dissolved to form a homogeneous solution. Reactions were carried out only when the mixture was homogeneous.

Reaction in Non-aqueous Reversed Micellar System: A sample solution was prepared by solubilizing an aqueous ATP solution in *n*-hexane using DAP (Fig. 1). When the amount of the aqueous solution added is too large for the DAP concentration, the aqueous solution is not solubilized completely, and when too small, DAP does not dissolve completely. The solubilized system was obtained in a range of $[H_2O]$/[DAP] ratio of 0.55—2.2 at DAP concentration of 0.2 M at 50 °C, and reactions were carried out only under these conditions at 50 °C.

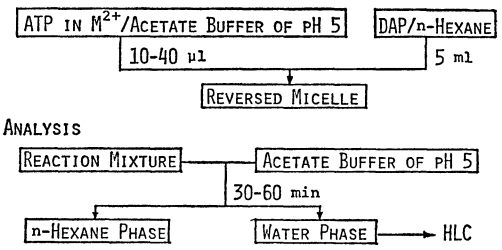

Fig. 1. Experimental procedure for the reversed micellar system.

Analysis. ATP and its hydrolysis products, *i.e.* ADP, adenosine 5′-monophosphate(AMP) and adenosine, were determined by a high speed liquid chromatography (hlc) under following conditions: column, Hitachi anion exchanger #2630; column temprature, 45 °C; mobile phase, 0.25 M NaCl in 0.01 M HCl solution; flow rate, 1.2 ml·min^{-1}; detector, Iatron UV photometer LC-1 at 254 nm. When *n*-hexane was used as a solvent, ATP and its hydrolysis products were extracted from the reaction mixture with water prior to hlc analysis. Under the condition that less than 10 per cent of initial amount of ATP was hydrolyzed, the hydrolysis product was mainly ADP, and the amount of AMP or adenosine produced was negligibly small. Therefore, the hydrolysis rate of ATP was determined from the change in ADP concentration in this range.

Other Measurements. UV spectra of pyridine 1-oxide were observed by a Hitachi spectrophotometer model EPS-3T using a thermostated cell. Aggregation numbers of DAP

in the reversed micellar system were estimated from the average molecular weights of micelles, which were measured by a Hewlett-Packard vapour pressure osmometer model 302B at 37 °C.

Results and Discussion

The rate of hydrolysis reaction of ATP in an aqueous solution obeys a pseudo first order rate kinetics for ATP concentration (Eq. (1));

$$\text{ATP} + \text{H}_2\text{O} \xrightarrow{k_{app}} \text{ADP} + \text{phosphoric acid}$$

$$-\frac{d[ATP]}{dt} = k^{\circ}_{app}[ATP] \quad (1)$$

where k°_{app} is a pseudo first order rate constant. The hydrolysis rate in the micellar system shows also a first order dependence on ATP concentration, and the pseudo first order rate constants, k^{m}_{app} in an aqueous micellar system and k^{r}_{app} in a reversed micellar system were determined experimentally.

ATP Hydrolysis in Aqueous Micellar Solutions. Effects of surfactants on k^{m}_{app} are shown in Table 1. The addition of an anionic surfactant, SDS, has little or no effect on k_{app}, but the addition of a cationic surfactant, CTAB, reduces it. Since the addition of tetramethylammonium bromide (TMAB) which does not form micelles shows no effect on the hydrolysis rate, the deceleration effect of CTAB is thought to be caused by the formation of its cationic micelles. Figure 2 shows the effect of CATB concentration on k^{m}_{app}. When the CTAB concentration is higher than 8×10^{-3} M, the value of k^{m}_{app} is reduced by half compared to k°_{app} and is almost independent of CTAB concentration.

Table 1. Hydrolysis rate constants of ATP in aqueous micellar solutions at 50 °C

Surfactant or salt added	$k_{app}\times10^{6}$ (s^{-1})	$k_{rel.}$
None	1.12	=1
SDS	1.04	0.92
CTAB	0.676	0.60
TMAB	1.09	0.97

ATP=2×10^{-3} M; Surfactant or salt=2×10^{-2} M; in pH 4.1 acetate buffer.

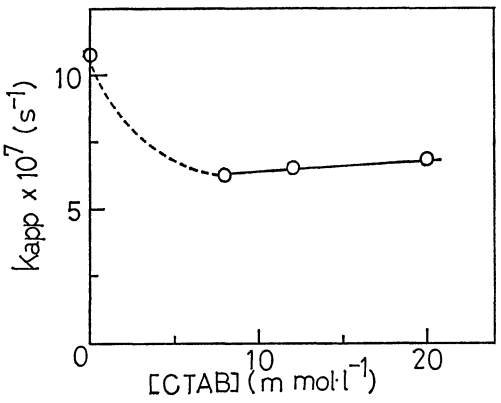

Fig. 2. Effect of CTAB concentration on the hydrolysis rate of ATP(2×10^{-3} M) at 50 °C in pH 4.1 acetate buffer.

The rate of hydrolysis could not be measured at lower CTAB concentration, where ATP did not dissolve uniformly.

The effect of addition of methanol to the reaction mixture with or without CTAB was examined, and result is shown in Fig. 3. With an increase in methanol content, the value of k^{m}_{app} in micellar solution becomes close to that of k°_{app}, and both the values are almost the same at methanol contents higher than about 50 v/v%, where CTAB micelles were destroyed almost completely.[6] This fact ensures that the effect of CTAB on hydrolysis rate is caused by its cationic micelles.

The effect of pH on hydrolysis rate both in aqueous and in CTAB micellar systems is given in Fig. 4. The value of pH of these solutions was adjusted by addition of a *dil* NaOH solution and determined by a glass electrode pH meter. Both of k°_{app} and k^{m}_{app} decrease as pH increases, and abrupt decreases are observed around pH 7 for k°_{app} and pH 6 for k^{m}_{app}. These pH values are nearly equal to those of the second dissociation of γ-phosphate of ATP in these systems (pk°_{2}= 7.1 and pk^{m}_{2}=6.1), which were determined from titration curves shown in the same figure. This result indicates that the hydrolysis rate is closely related with

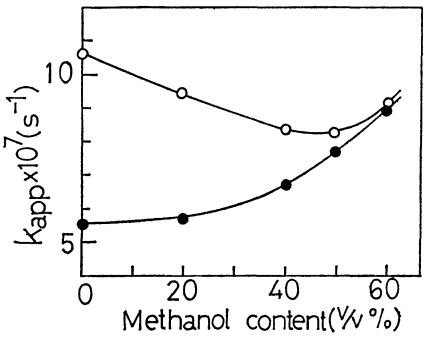

Fig. 3. Effect of methanol on the hydrolysis rate of ATP (2×10^{-3} M) both in aqueous (○), and in CTAB (1.2×10^{-2} M) micellar (●) solutions at 50 °C.

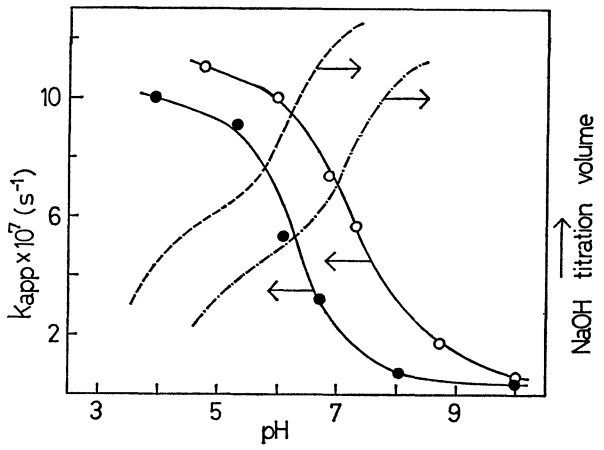

Fig. 4. Effect of pH on the hydrolysis rate of ATP (2×10^{-3} M) in aqueous (○), and in CTAB (1.2×10^{-2} M) micellar (●) solutions; and titration curves of ATP in aqueous (·—·), and in CTAB (1.2×10^{-2} M) micellar (----) solutions; at 50 °C.

the near environment of ATP or the dissociation state of ATP. Furthermore, it is to be noted that shifting the curve of k°_{app} *vs.* pH to the lower pH side by about 1 makes it similar to that of k^{m}_{app} *vs.* pH. This result might imply that the pH value at the reaction site in micellar solution is higher by about 1 than that determined by a pH meter, which measures the pH value in the bulk aqueous phase.[7] This leads to the following interpretation of the result. ATP molecules exist at the interface between water and micelle, where hydroxide ions are accumulated by its cationic character to give a rise in pH. The deceleration effect of CTAB micellar system on hydrolysis rate of ATP is owing to this accumulation effect at micellar interface.

ATP Hydrolysis in Reversed Micellar Systems. The hydrolysis of ATP was also examined in a reversed micellar system in nonpolar medium. *n*-Hexane was used as a nonpolar solvent and DAP as an oil-soluble surfactant. ATP, which is insoluble in organic solvents, could be solubilized in *n*-hexane by addition of a small amount of water in the presence of DAP to form a reversed micellar system. By assuming that ATP molecules would exist in the core of reversed micelle or, in other words, in the water phase dispersed in *n*-hexane, the rate constant k^{r}_{app} in this system was obtained by a similar procedure to that in aqueous systems. The value of k^{r}_{app} thus obtained was proportional to the DAP concentration and inversely proportional to the water concentration in the range examined; the result could be represented by the expression;

$$-\frac{d[ATP]}{dt} = k^{r}_{app}[ATP] = k'\frac{[DAP]}{[H_2O]}[ATP] \qquad (2)$$

as shown in Fig. 5. The value of k^{r}_{app} is 1—5 times larger than that of k°_{app}; that is, ATP hydrolysis is enhanced in the reversed micellar system.

Effects of Metal Ions. It is known that Mg^{2+} and Ca^{2+} play an important role in the enzymatic hydrolysis of ATP in living systems. In this respect, the effects of these ions on hydrolysis of ATP in aqueous micellar and reversed micellar systems were examined by adding these chlorides into the systems. The values of k°_{app} and k^{m}_{app} are little affected, but that of

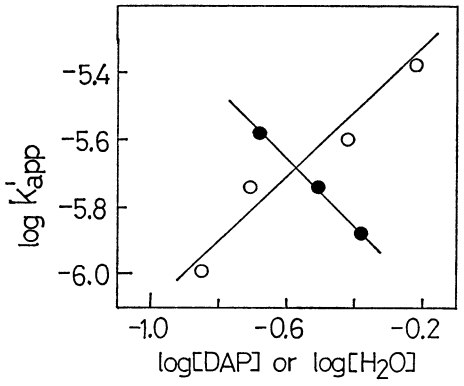

Fig. 5. Dependences of the hydrolysis rate of ATP (6×10^{-4} M) both on DAP concentration (○) where water concentration was 3.33×10^{-1} M, and on water concentration (●) where DAP concentration was 2×10^{-1} M; in *n*-hexane at 50 °C.

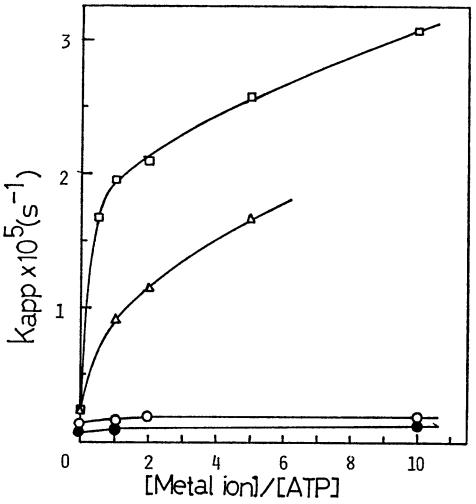

Fig. 6. Effect of divalent metal ions on hydrolysis rate of ATP (6×10^{-4} or 2×10^{-3} M) at 50 °C; (□): M^{2+}, reversed micellar system in *n*-hexane (DAP= 2×10^{-1} M, $H_2O=3.33\times10^{-1}$ M); (△): Ca^{2+}, reversed micellar system in *n*-hexane (DAP=2×10^{-1} M, $H_2O=3.33\times10^{-1}$ M); (○): Mg^{2+}, aqueous solution (pH=4.1); (●): Mg^{2+}, aqueous micellar system (CTAB=1.2×10^{-2} M, pH=4.1).

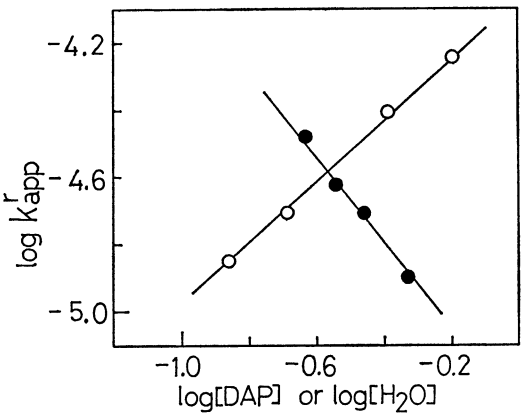

Fig. 7. Dependences of the hydrolysis rate of ATP (6×10^{-4} M) in the presence of Mg^{2+} (1.2×10^{-3} M) both on DAP concentration (○) where water concentration was 3.33×10^{-1} M, and on water concentration (●) where DAP concentration was 2×10^{-1} M; in *n*-hexane at 50 °C.

k^{r}_{app} markedly increases with an increase in the amount of Mg^{2+} added, and the value of k^{r}_{app} levels off at about Mg^{2+}/ATP=1 as are shown in Fig. 6. This indicates that the hydrolysis rate is enhanced by the formation of 1 : 1 complex of Mg^{2+}–ATP. Addition of Ca^{2+} also shows a rate enhancement in the reversed micellar system though its effect is smaller than that of Mg^{2+}.

The effect of amounts of DAP and water on k^{r}_{app} was examined at Mg^{2+}/ATP=2. The result shows that the hydrolysis rate obeys Eq. (2) as is shown in Fig. 7.

Rate Enhancement in Reversed Micellar System. The hydrolysis reaction of ATP is enhanced in reversed micellar system, and especially when Mg^{2+} or Ca^{2+} is added. The hydrolysis rate is followed by Eq. (2)

whether the divalent cation is added or not. This result suggests that the micellar reaction mechanism itself is not affected by the presence of divalent cation.

The aggregation number of DAP in the reversed micellar system was measured by a vapor pressure osmometer and the result is shown in Table 2. The aggregation number is small and therefore the number of water molecules solubilized in the core of the reversed micelle is very small. So, it is hard to consider the ion condensation at the interface as being observed in the aqueous cationic micellar system. Some other factors must contribute to the rate enhancement.

It is important to note that k^{r}_{app} depends on [DAP]/[H_2O] ratio. Menger *et al.* measured the Z value of water phase solubilized in octane by an anionic surfactant, di-2-ethylhexyl sodium sulfosuccinate, by using pyridine 1-oxide and 2-toluidinylnaphthalene-6-sulfonate as probes, and observed that the value depends on [H_2O]/[Surfactant] ratio.[8] We also measured the polarity of water solubilized in DAP reversed micelles in *n*-hexane by using pyridine 1-oxide as a probe. The change in absorbance maxima of pyridine 1-oxide near 260 nm is given in Fig. 8 as a function of [H_2O]/[DAP] ratio. The polarity of water in the core of the reversed micelle shows a good linear dependence on [H_2O]/[DAP] ratio, and lies midway between those of ethanol and chloroform at [H_2O]/[DAP]=0.55. On the other hand, Nelson *et al.* observed that the hydrolysis rate of ATP was enhanced in aqueous solution of aprotic polar solvent such as dimethyl sulfoxide, dioxane or formamide.[3] Considering them altogether, the rate enhancement of ATP hydrolysis in the DAP reversed micellar system is supposed to be caused by lowering in the polarity of water phase in the core of the reversed micelle, and the [DAP]/[H_2O] term in Eq. (2) may represent the degree of lowering in the polarity.

Table 2. Aggregation number of DAP in the reversed micellar solutions at 37 °C

[H_2O]/[DAP] ratio	Aggregation number
0.55	4.56
1.10	4.53
1.75	4.61
2.20	4.66
2.75	5.23

DAP=2×10^{-1} M; in *n*-hexane.

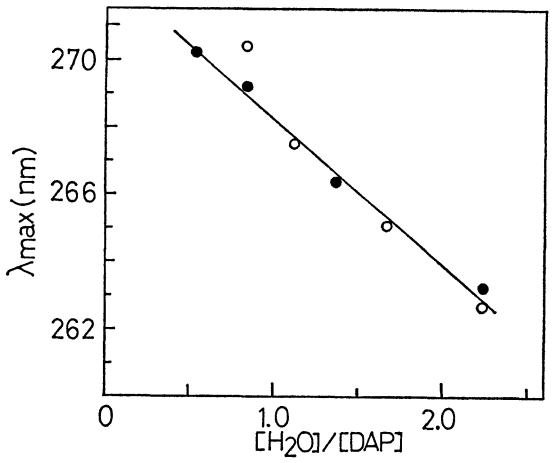

Fig. 8. Dependences of the absorbance maxima (λ_{max}) of pyridine 1-oxide (7.7×10^{-5} M) on [H_2O]/[DAP] ratio in reversed micellar system in *n*-hexane at 50 °C; (○): DAP concentration was 2×10^{-1} M at various water concentrations; (●): water concentration was 3.33×10^{-1} M at various DAP concentrations. λ_{max} of pyridine 1-oxide in various solvents was reported; 254.4 nm in water, 263.3 nm in methanol, 265.1 nm in ethanol, and 276.1 nm in chloroform.[9]

The active site of enzyme is said to be the polar site formed by various functional groups in the nonpolar medium. The reaction environment of enzyme resembles in a sense to that of the reversed micellar system in nonpolar solvent.

It is well known that a divalent cation, most often Mg^{2+} or Ca^{2+}, is required to activate ATPase in living system, but these metal ions show low or almost no activity in aqueous nonenzymatic hydrolysis of ATP. It is interesting that these metal ions are effective only in the reversed micellar system. Nelson *et al.* also observed that Mg^{2+} was much effective on ATP hydrolysis and Tagaki *et al.* on hydrolysis of phenyl phosphosulfate both in less polar media.[3,10]

Mg^{2+} and Ca^{2+} are hard acids and, therefore, have a stronger interaction with phosphate, which is a base harder than adenine ring. Moreover, it was found that Mg^{2+} ion, a harder acid than Ca^{2+}, is more effective than Ca^{2+} in the reversed micellar system. It was suggested from this consideration that the effect of these ions on ATP hydrolysis is caused by the neutralization of negative charges on phsophate part of ATP. By supposing that the hydrolysis of ATP proceeds *via* nucleophilic mechanism, this reasoning would be plausible. On this respect, it is interesting to note that the mechanism of γ-phosphate splitting of ATP by ATPase is nucleophilic.

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