C-H bonds (entries 7, 8). Extraneous steric effects may substantially change the reaction course. Thus only γ-lactone products were obtained from both neomenthyl and menthyl diazoacetoacetates where insertion reaction occurred away from the neighboring isopropyl group (entries 9, 10). In two of the cases (entries 7, 9) five-membered ring formation is relatively more pronounced compared to the cases with corresponding methyl diazomalonates.

Use of insertion reactions of diazoacetoacetate and diazomalonate in organic synthesis will be the subject of future studies in these laboratories.

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Metallomicelle-Nucleic Acid Interaction: Copper(II) Dodecylsulfate-Catalyzed RNA Cleavage

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Numerous instances of catalytic hydrolysis of the sugar-phosphate backbone of RNA (especially $tRNA^{Phe}$) by several divalent metal ions such as Mg^{2+} [1], Zn^{2+} and Pb^{2+} [2], and also by Eu^{3+} [3] are known. The metal ion-catalyzed cleavage of $tRNA^{Phe}$ is thought to be an intramolecular version of a metallo-enzyme-catalyzed reaction[4]. Catalytic activities of most nucleases[5] and RNA catalysts[6] are also dependent on metal ions. On the other hand, studies as for the micellar models of hydrolytic metalloenzymes have been introduced very recently[7-11]. We can anticipate the alteration of the hydrolytic activity of the metal ions by being associated with the surfactant molecules in the micelle.

Metallomicelles formed from long-chained Cu^{2+} complexes had remarkable hydrolytic catalysis activity against phosphate triesters, diesters, and other phosphorus compounds[10]. The high hydrolytic catalysis activity of the metallomicelles may be due to enhanced electrophilicity of micellized metal and consequently the increased activity of the metal-bound water[10]. These previous reports prompted us to test any hydrolytic activity of some metallomicelles against nucleic acids. In this paper we show ribonuclease-like (RNase-like) activity of bivalent metal ion (Cu^{2+}, Mg^{2+}, and Pb^{2+}) complexes of sodium dodecyl sulfate (SDS, $CH_3(CH_2)_{11}SO_4^-Na^+$), which form metallomicelles $[CH_3(CH_2)_{11}SO_4^-]_2M^{2+}$, $M(DS)_2$.

Baker's yeast RNA type XI purchased from Sigma was used as substrate. Sodium dodecyl sulfate purchased from Wako Pure Chemical Industries, LTD was selected as surfactant for making micelle systems.

Buffer solution: Tris, despite its negligible interaction with the metal ions Mg^{2+} and Cu^{2+}, was abolished, since it interacts with the DS^-. Acetate buffer (pH 5.0), having no such interactions was used as buffer.

Assay of RNase-like activity: Among various assay methods, the method monitoring the activity producing the hydrolyzed soluble fraction by measuring the spectrophotometric absorbance at 260 nm of wavelength after precipitating unreacted substrate RNA was adopted. As the precipitation agent for unreacted RNA, perchloric acid containing lanthanum nitrate[12] was used. This technique gives reproducible results with low blanks and without overlapping light absorption in the region of 260 nm wavelength of RNA absorption peak, while inclusion of uranyl acetate instead of lanthanum nitrate affects the absorption peak of RNA at 260 nm. The biomimetic hydrolytic reaction was initiated by the addition of 1.5 m*l* of the RNA (substrate) solution to the equal volume of 8×10^{-5} M $Cu(DS)_2$ solution (the concentration far exceeding CMC, critical micelle concentration). The reaction mixture was well mixed and incubated at 25℃ or 60℃ for a difinite period of time (usually 7 hours). The reaction was terminated by the addition of 2 m*l* precipitant (perchloric acid/lanthanum nitrate) to 1 m*l* portion of the reaction mixture. The precipitant added reaction mixture was left alone for 40 min, and then centrifuged for 10 min, at 6000 rpm to remove the precipitates of unreacted RNA and $M(DS)_2$. The hydrolytic ribonuclease-like activity of the metallomicelles was determined by the measurement of the absorbance of the supernatant fraction at 260 nm by UVICON 860 Spectrophotometer after five-fold dilution with distilled water. In the absorbance measurement, the blank solution was that of the same composition as the reaction mixture except for the omission of the substrate RNA and treated in the same way along with the reaction mixture.

The buffer system for the RNA hydrolysis by Cu (II) dode-

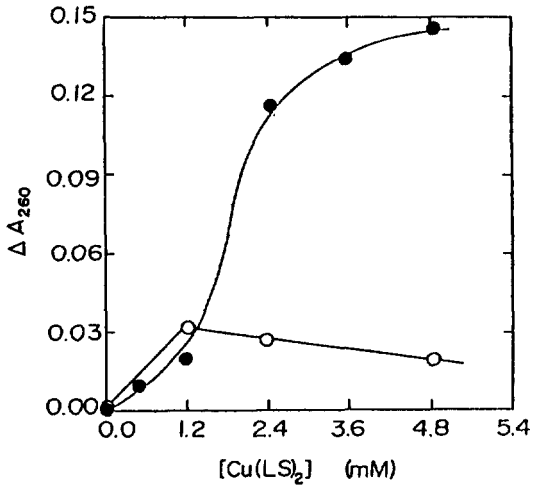

Figure 1. $Cu(DS)_2$ concentration-dependent rate of liberation of acid-soluble products from RNA. Three ml of reaction mixture containing (0.065 mg/ml) substrate RNA was incubated with various concentrations of $Cu(DS)_2$ at 25℃ (○—○) and 60℃ (●—●) respectively. Experimental conditions are described in materials and methods.

cylsulfate [$Cu(DS)_2$] was 0.05 M acetate buffer pH 5.0. The use of other buffer solutions of higher pH tended to cause the precipitation of the metal ion hydroxide. The RNA hydrolytic activity of the $Cu(DS)_2$ *vs* $Cu(DS)_2$ concentration appears in Figure 1. The metallomicelles have much higher activity at 60℃ than 25℃. The graph of the activity at 25℃ levels down earlier than that of 60℃. It appears that $Cu(DS)_2$ concentrations where the climbing graphs level down correspond roughly to their respective CMC: 2.6 mM at 60℃ and 1.2 mM at 25℃. These data show that the RNase-like activity of $Cu(DS)_2$ is dependent not only on the $Cu(DS)_2$ concentration but also on temperature. The temperature-composition phase diagrams for SDS in 100 mM NaCl and in $Cu(DS)_2$ are expected to be similar, having similar micellar properties such as CMC of 1.3 mM[13] and 1.2 mM[14] respectively under the same conditions. At the $Cu(DS)_2$ concentration of 4 mM used, the concentration much greater than the CMC at 60℃ (2.6 mM) and far above the CMT (critical micellar temperature), the $Cu(DS)_2$ system is expected to exist in micellar solution; and at 25℃, the temperature around CMT, the system may exist in micellar solution but partly in crystalline suspension rather than as monomer solution, since the $Cu(DS)_2$ concentration is above the CMC. Now looking at Figure 1 which shows the dependence of RNase-like activity of $Cu(DS)_2$ system on the $Cu(DS)_2$ concentration, with the data of the temperature-composition phase diagram in mind, we can notice that $Cu(DS)_2$ can have RNase-like activity only in the phase of micellar solution. To attain the micellar solution phase, the temperature should be above the CMT and the $Cu(DS)_2$ concentration above the CMC. Monomers rather than micellar solution may exist above the temperature of CMT but below the CMC. The graph of 60℃ rises rapidly up to the $Cu(DS)_2$ concentration of around 2.4 mM, which is close to the CMC at 60℃, and from which the climbing rate slows down. The upward curvature indicates that the active components consist of aggregates (micellar systems) of monomer metallosurfactant molecules which are inactive

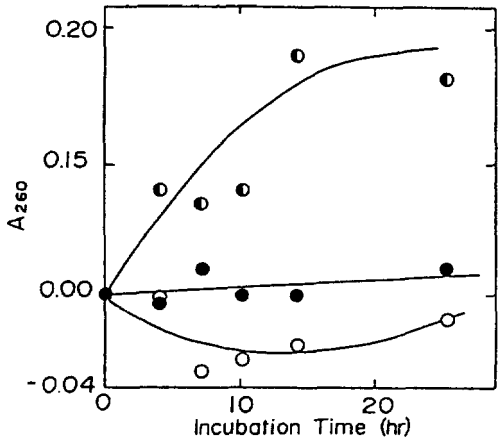

Figure 2. Time course of reaction of hydrolytic RNA cleavage at 25℃ by the metallomicelles of $Cu(DS)_2$ (◐) along with the tests in case of free Cu^{2+} (●) and free SDS (○) instead of $Cu(DS)_2$. Other experimental conditions and methods are the same as those of Figure 1.

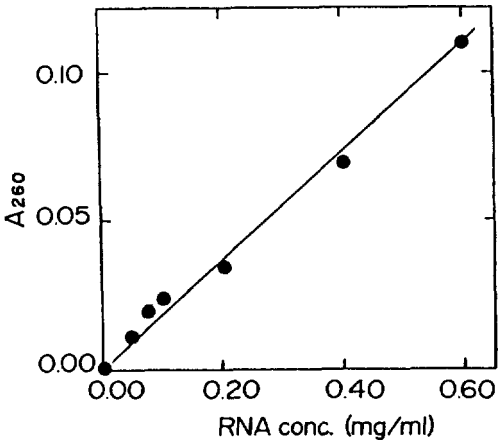

Figure 3. Effect of substrate concentraction on the RNase-like activity of the metallomicelle of $Cu(DS)_2$ (1.2×10^{-3} M) at 25℃. Other experimental conditions and methods are the same as those of Figure 1.

singly. Since increasing concentration to CMC will favor cooperative mecelle formation the active micelle concentration and thus the RNase-like activity curve will bend upwards. Thus this profile of RNase-like activity rise may reflect the profile of catalytic micelle concentration increase as the concentration of $Cu(DS)_2$ increases. The upward curvature of this profile resembles the enzyme systems where the active enzyme consists of a complex of subunits which are inactive singly[15]. The CMC is dependent on temperature[16]. In the 25℃ graph the earlier formation of activity peak, albeit low, may be due to lower CMC at the lower temperature and the slight decrease in the activity with the increase of the $Cu(DS)_2$ concentration may be due to crystalline suspension formation from the added $Cu(DS)_2$.

We tested RNase-like activity of Cu^{2+} and SDS alone respectively, and the data are shown in Figure 2. We can notice that Cu^{2+} or SDS alone does not have the RNA-cleavage activity, whereas $Cu(DS)_2$ in the form of micellar solution has the activity. The activity slows down in longer incubation.

We, then, tested the substrate concentration dependent RNase-like activity of the $Cu(DS)_2$ micellar solution. The activity increased linearly as the concentration of the substrate RNA was increased up to the concentration of 0.60 mg/ml tested (Figure 3).

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Correlation Between Cross-Interaction Constant and Transition-State Imbalance

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Cross-interaction constant and transition state imbalance are two important concepts in organic reaction mechanisms. Here we report a close relationship between the two.

Cross-interaction constants ρ_{XZ} or β_{XZ} (Eq. 1) defined by a Taylor expansion of log k_{XZ} up to second order around $\sigma_X=\sigma_Z=0$ or $\Delta pK_X=\Delta pK_Z=0$ where X and Z are substituents in the nucleophile, substrate or leaving group, and $\Delta pK_X=\Delta pK_a^X-\Delta pK_a^H$ *etc.*, and neglecting the pure second-order terms, ρ_{XX}, β_{ZZ} *etc.*, have proved to be a useful measure of transition state (TS) structure[1]. The magnitude of β_{XZ} represents the intensity of direct interaction

$$\log\ (k_{XZ}/k_{HH})=\rho_X\sigma_X+\rho_Y\sigma_Y+\rho_{XY}\sigma_X\sigma_Y \quad (1a)$$

$$=\beta_X\Delta pK_X+\beta_Z\Delta pK_Z+\beta_{XZ}\Delta pK_X\Delta pK_Z \quad (1b)$$

between two reaction centers, R_X and R_Z, so that the $|\beta_{XZ}|$ is related inversely to the distance between the two reaction centers under a set reaction condition[1].

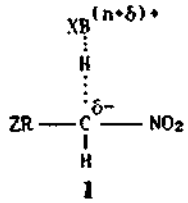
In proton transfers involving carbon acids (Eq. 2) or nucleophilic additions to olefins (Eq. 3) with a strong electron withdrawing substituent, *e.g.*, NO_2, that stabilizes the negative charge, structural and solvent reorganizations in forming resonance stabilized carbanion are often

$$ZRH_2CNO_2+XB^n \rightleftharpoons ZR-\underset{H}{\overset{|}{C}}=\underset{O}{\overset{O}{N}}- + BH^{n+1} \quad (2)$$

$$ZRCH=CHNO_2+XB^n \rightleftharpoons ZR\underset{XB^{n+1}}{\overset{H}{C}}-\underset{H}{\overset{|}{C}}=\underset{O}{\overset{O}{N}}- \quad (3)$$

found to lag behind the bond changes in the TS[2]. This phenomenon referred to as "imbalance", (I), manifests itself by an inequality between the Brönsted β_X (variation of base; denoted as β_B for proton transfer and β^n_{nuc} for nucleophilic addition[3]) and β_Z (variation of substrate; α_{CH} for proton transfer and α^n_{nuc} for nucleophilic addition[3]), $I=\beta_Z-\beta_X$. The inequality $\beta_Z>\beta_X$ is known to arise from an exalted β_Z value relative to the "normal" β_X representing an approximate measure of the degree of proton transfer or progress of adduct formation in the TS.

The enhanced value of β_Z over β_X is observed because in the TS, **1** and **2**, the negative charge is mainly on carbon in contrast to the delocalized anion on the nitro group in the product ion (Eq. 2 and 3).



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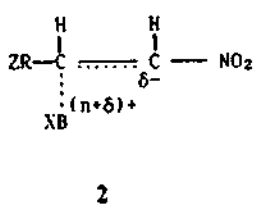

2

The lag in the delocalization of negative charge in the TS will result in the excess electron density on reaction center or the adjacent carbon over that would have if the resonance development were normal in the TS, and as a result the interaction between the two reaction centers will become enhanced *i.e.*, $|\beta_{XZ}|$ will become enhanced due to the imbalance. This enhanced $|\beta_{XZ}|$ induced by the imbalance, $|\beta_{XZ}{}_{(imb)}|$, is thus expected to vary proportionally to the magnitude of the imbalance, $|I|$.