

Copper(II)-Catalyzed Transamination of Hydrophobic Pyridoxamine with Pyruvic Acid in Functionalized Bilayer Vesicles†

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The copper(II)-catalyzed transamination of 2-methyl-3-hydroxy-4-aminomethyl-5-(dodecylthiomethyl)pyridine (C_{12} SPM) with sodium pyruvate was investigated in an aqueous medium at pH 6.8, μ 0.10 (KCl), and $30.0 \pm 0.1^\circ\text{C}$ in the presence of molecular aggregates of N,N -ditetradecyl- N^α -[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5\text{Ala}2C_{14}$), N,N -ditetradecyl- N^α -[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5\text{His}2C_{14}$), or hexadecyltrimethylammonium bromide (CTAB). The reaction afforded the corresponding pyridoxal analogue (C_{12} SPL) and alanine as the final products upon addition of edta which liberates the copper(II) ion from the coordination sites of the aldimine Schiff-base. The coordination interaction between the copper(II) ion and C_{12} SPM, which takes place prior to the transamination, was clarified by electronic spectroscopy. The reactivity of the 2:1 (ketimine: Cu^{II}) complex was found to be much larger than that of the 1:1 complex in the molecular assemblies of $N^+C_5\text{Ala}2C_{14}$ and CTAB, and the formation of the former species was more pronounced in the $N^+C_5\text{Ala}2C_{14}$ vesicle. The bilayer vesicle formed with $N^+C_5\text{His}2C_{14}$ allowed the formation of the 1:1 complex in preference to that of the 2:1 complex, and the coordination-free imidazolyl group of the amphiphile effectively catalyzed the isomerization as a general base.

Pyridoxal-5'-phosphate (PLP) is known to catalyze various transformation reactions of amino acids such as transamination, elimination, racemization, and decarboxylation as the coenzyme in biological systems.¹⁾ Although most of the PLP-dependent enzymatic reactions can be simulated by nonenzymatic systems,²⁾ such nonenzymatic reactions are much less efficient than the corresponding enzymatic ones with respect to reactivity, substrate specificity, and reaction selectivity. In order to improve the catalytic efficiency of model systems for the PLP-dependent enzymes, micellar surfactants,³⁾ synthetic polymers,⁴⁾ and macrocyclic compounds⁵⁾ have been utilized in recent years to simulate the functions of apoenzymes. We have reported previously that a bilayer membrane composed of a synthetic peptide lipid can provide a reaction field favorable for the transamination of PLP with a hydrophobic amino acid.⁶⁾

In the present work, we prepared hydrophobic vitamin B₆ analogues, C_{12} SPM and C_{12} SPL, and investigated the reaction of C_{12} SPM with pyruvate in single-walled vesicles of $N^+C_5\text{Ala}2C_{14}$ and

$N^+C_5\text{His}2C_{14}$ as effected by the copper(II) ion. The kinetic behavior of the transamination reaction was analyzed in the light of metal-coordination modes observed for C_{12} SPM and its ketimine Schiff-base with the copper(II) ion. The kinetic effect of single-walled vesicles formed with $N^+C_5\text{His}2C_{14}$ on the transamination is to be discussed in view of the base catalysis of the histidyl residue.

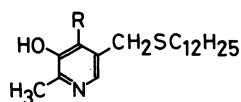
Experimental

Spectroscopic data were taken on a Union Giken SM-401 UV-visible spectrophotometer and a Hitachi R-24B NMR spectrometer. pH-Measurements were carried out with a Beckman expandomatic SS-2 pH meter equipped with a Metrohm EA-125 combined electrode after calibration with a combination of appropriate standard aqueous buffers. Melting points were measured with a Yanaco MP-S1 apparatus (hot-plate type). Elemental analyses were performed at the Microanalysis Center of Kyushu University.

Hexadecyltrimethylammonium bromide (CTAB) of Nakarai Chemicals was recrystallized from ethanol, mp $237\text{--}239^\circ\text{C}$ (decomp). N,N -Ditetradecyl- N^α -[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5\text{Ala}2C_{14}$) and N,N -ditetradecyl- N^α -[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5\text{His}2C_{14}$) were prepared as described elsewhere.^{6,7)} 2-Methyl-3-hydroxy-4-aminomethyl-5-(dodecylthiomethyl)pyridine dihydrobromide (C_{12} SPM·2HBr) and 2-methyl-3-hydroxy-4-formyl-5-(dodecylthiomethyl)pyridine (C_{12} SPL) were prepared as described below.

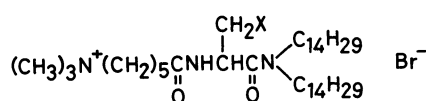
2-Methyl-3-hydroxy-4-aminomethyl-5-(bromomethyl)pyridine Dihydrobromide (PMBr). Pyridoxamine dihydrochloride (1.0 g, 4.2 mmol) dissolved in 48% aqueous hydrobromic acid (25 ml) was refluxed for 2 h. After the mixture was evaporated to dryness *in vacuo*, the crude product was dissolved in methanol and purified by reprecipitation with ether; yield 1.3 g (81%), mp $265\text{--}270^\circ\text{C}$ (decomp).

2-Methyl-3-hydroxy-4-aminomethyl-5-(dodecylthiomethyl)pyridine Dihydrobromide (C_{12} SPM·2HBr). A solution of 1-dodecanethiol (530 mg, 2.6 mmol) in dry ethanol (5 ml) was added dropwise to 50 mg of sodium metal dissolved in dry ethanol (5 ml) with stirring under nitrogen atmosphere. PMBr (400 mg, 1.0 mmol) dissolved in dry ethanol (50 ml) was added dropwise to the resulting mixture with stirring under nitrogen at 0°C . The mixture was evaporated to dryness *in*



C_{12} SPM (R = CH_2NH_2)

C_{12} SPL (R = CHO)



$N^+C_5\text{Ala}2C_{14}$ (X = H)

$N^+C_5\text{His}2C_{14}$ (X =)

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vacuo, and the residue was recrystallized from methanol. The crude product dissolved in methanol was treated with 48% aqueous hydrobromic acid, evaporated to dryness, and purified by gel-filtration chromatography on columns of Toyopearl HW-40 fine and Sephadex LH-20 in this sequence with methanol as an eluant to afford a white solid: yield 0.17 g (33%), mp 193–194 °C (decomp); ¹H-NMR (CD₃OD, TMS) δ =0.88 [3H, t, S(CH₂)₁₁CH₃], 1.27 [20H, m, SCH₂(CH₂)₁₀CH₃], 2.50 [2H, t, SCH₂(CH₂)₁₀CH₃], 2.67 [3H, s, 2-CH₃ on pyridine ring], 3.96 [2H, s, CH₂S(CH₂)₁₁CH₃], 4.39 [2H, s, CH₂NH₃⁺], and 8.16 [1H, s, 6-H on pyridine ring].

Found: C, 47.15; H, 7.72; N, 5.66%. Calcd for C₂₀H₃₆N₂OS·2HBr: C, 46.70; H, 7.45; N, 5.45%.

2-Methyl-3-hydroxy-4-formyl-5-(dodecylthiomethyl)pyridine (C₁₂SPM). A mixture of potassium hydroxide (1.37 g, 9.8 mmol), C₁₂SPM·2HBr (0.10 g, 0.19 mmol), α -oxoglutaric acid (1.46 g, 10 mmol), and zinc nitrate (0.116 g, 0.39 mmol) in methanol (200 ml) was stirred for 24 h at room temperature, and evaporated to dryness *in vacuo*. The residue was suspended in 2 mol dm⁻³ aqueous hydrochloric acid (100 ml), and extracted with chloroform (3 × 50 ml). The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness *in vacuo*. The crude product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as an eluant to afford a pale yellow oil: yield 29 mg (39%); ¹H-NMR (CDCl₃, TMS) δ =0.89 [3H, t, S(CH₂)₁₁CH₃], 1.29 [20H, m, SCH₂(CH₂)₁₀CH₃], 2.35 [2H, br s, SCH₂(CH₂)₁₀CH₃], 2.51 [3H, s, 2-CH₃ on pyridine ring], 3.90 [2H, s, CH₂S(CH₂)₁₁CH₃], 7.87 [1H, s, 6-H on pyridine ring], and 10.45 [1H, s, CHO].

Found: C, 68.06; H, 9.46; N, 4.01%. Calcd for C₂₀H₃₃NO₂S: C, 68.34; H, 9.46; N, 3.98%.

Kinetic Measurements. The reaction medium was prepared as follows. (i) An appropriate amount of the stock solution of one of the amphiphiles (N⁺C₅Ala2C₁₄, N⁺C₅His2C₁₄, and CTAB) in ethanol was placed in a glass vessel, and evaporated *in vacuo* to remove the solvent completely. An aqueous HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) buffer (2.5 mmol dm⁻³, pH 6.8) containing 0.1 mol dm⁻³ potassium chloride was added to the residue. (ii) An aqueous dispersion of the amphiphile thus prepared was sonicated for 2 min with a probe-type sonicator at 30-W power (W-220F, Heat Systems-Ultrasonics). The resulting vesicle solution was sufficiently clear and gave good kinetic reproducibility. Each run was initiated by adding 20 μ l of an aqueous solution of C₁₂SPM (5.0 × 10⁻⁵ mol dm⁻³), an appropriate quantity of an aqueous solution of Cu(ClO₄)₂ which was standardized by conventional chelatometric titration (1.0 × 10⁻² mol dm⁻³), and 100 μ l of a freshly prepared aqueous solution of sodium pyruvate (0.1 mol dm⁻³) to 2.0 ml of the reaction medium which was pre-equilibrated at 30.0 ± 0.1 °C in a thermostated cell set in the spectrophotometer.

Results and Discussion

Spectral Change in the Course of Transamination.

The transamination reaction of C₁₂SPM with sodium pyruvate, as effected by the presence of single-walled vesicles of N⁺C₅Ala2C₁₄ and N⁺C₅His2C₁₄ as well as by CTAB micelles, was followed spectrophotometrically in the aqueous HEPES buffer at pH 6.8, μ 0.10 (KCl), and 30.0 ± 0.1 °C. Figure 1 shows the spectral change observed along the progress of transamination in the presence of the N⁺C₅Ala2C₁₄ vesicle. The absorption spectrum of C₁₂SPM in the single-walled vesicle shows a maximum at 293 nm and a shoulder at 315 nm. This indicates the presence of two species which are different

in protonation site from each other (Scheme 1).^{8,9} The charged form (PM₁) was primarily observed in dioxane–water (1:9 v/v), and the fraction of the neutral

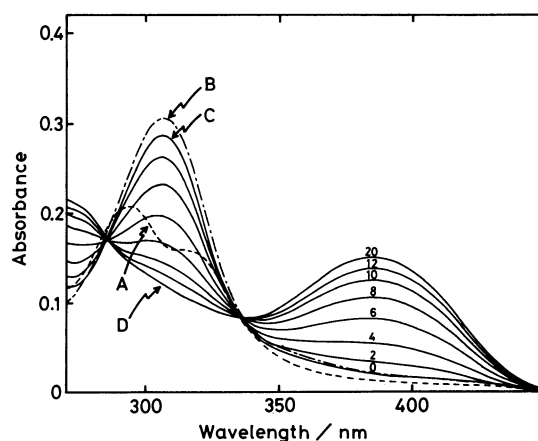
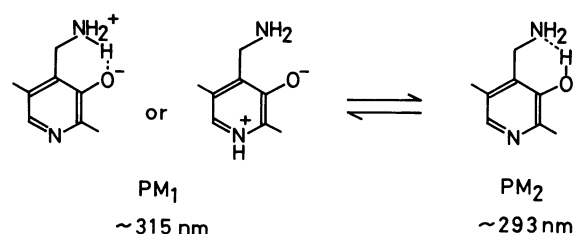


Fig. 1. Spectral change along the progress of transamination of C₁₂SPM (5.0 × 10⁻⁵ mol dm⁻³) with sodium pyruvate (5.0 × 10⁻³ mol dm⁻³) in the presence of Cu(ClO₄)₂ (5.0 × 10⁻⁵ mol dm⁻³) and N⁺C₅Ala2C₁₄ vesicle (1.0 × 10⁻³ mol dm⁻³) in an aqueous HEPES buffer (2.5 × 10⁻³ mol dm⁻³) at pH 6.8, μ 0.10 (KCl), and 30.0 ± 0.1 °C: A, C₁₂SPM alone; B, Cu^{II}–C₁₂SPM species; C, Cu^{II}–ketimine species; D, Cu^{II}–aldimine species. Numerals refer to reaction periods (in min) for the ketimine–aldimine isomerization.



Scheme 1.

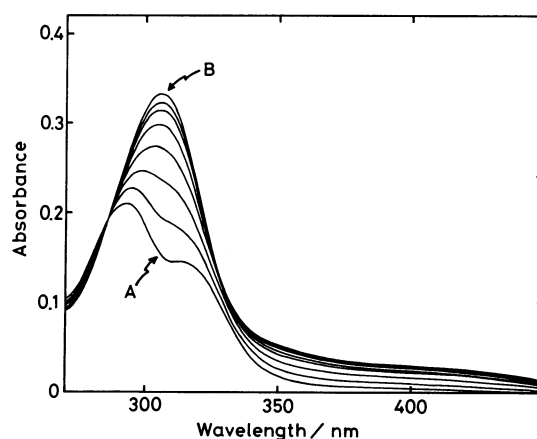


Fig. 2. Electronic absorption spectra of C₁₂SPM (5.0 × 10⁻⁵ mol dm⁻³) in an aqueous HEPES buffer (2.5 × 10⁻³ mol dm⁻³) at pH 6.8, μ 0.10 (KCl), and 30.0 ± 0.1 °C in the presence of N⁺C₅Ala2C₁₄ vesicle (1.0 × 10⁻³ mol dm⁻³) and varying amounts of Cu(ClO₄)₂: 0, 5.1 × 10⁻⁶, 1.0 × 10⁻⁵, 1.5 × 10⁻⁵, 2.0 × 10⁻⁵, 2.5 × 10⁻⁵, 3.0 × 10⁻⁵, and 5.0 × 10⁻⁵ mol dm⁻³ (read from A to B).

form (PM₂) increased with an increase in dioxane content. The microenvironmental polarity for the pyridoxamine moiety of C₁₂SPM in the aggregates was evaluated from the relative intensities of the absorption maxima for these species. As a result, both vesicular and micellar systems provide a microenvironment equivalent to that provided by dioxane–water (1:1 v/v) (Kosower's Z-value, 87).¹⁰ Addition of Cu(ClO₄)₂ resulted in an immediate shift of the absorption maximum to 306 nm along with an increase in intensity as shown in Fig. 2. This spectral change is analogous to that observed for the formation of the Cu^{II}–pyridoxamine complex in methanol⁹ and attributed to the formation of the Cu^{II}–C₁₂SPM complex (1 in Scheme 2). Subsequently, the ketimine Schiff-base complex (2 in Scheme 2), having an absorption maximum at 305 nm, was formed instantaneously upon addition of sodium pyruvate to the solution. The intensity of this peak decreased with time, isosbestic points being observed at 286 and 335 nm. A new absorption band appeared at 385 nm and increased in

intensity as the reaction proceeded. The absorption spectrum observed at the final stage of reaction was identical with that of the copper complex of the aldimine Schiff-base of C₁₂SPL formed with alanine (3 in Scheme 2). When an aqueous solution of tetrasodium ethylenediaminetetraacetate (edta; 10-fold molar excess over Cu^{II} ion) was added to the final solution, the spectrum changed immediately indicating the formation of the metal-free aldimine Schiff-base of C₁₂SPL formed with alanine. This Schiff-base was hydrolyzed to C₁₂SPL and alanine completely within 1 h. The isomerization of the ketimine Schiff-base to the corresponding aldimine was not observed in the absence of Cu(ClO₄)₂. Similar reaction behavior was found in other amphiphile systems of the present study. Table 1 summarizes the spectral parameters for various species formed in the present amphiphile systems.

In the light of these observations, the overall reaction pathway is explicitly given in Scheme 2. Matsumoto *et al.* reported that the reaction of pyridoxamine with

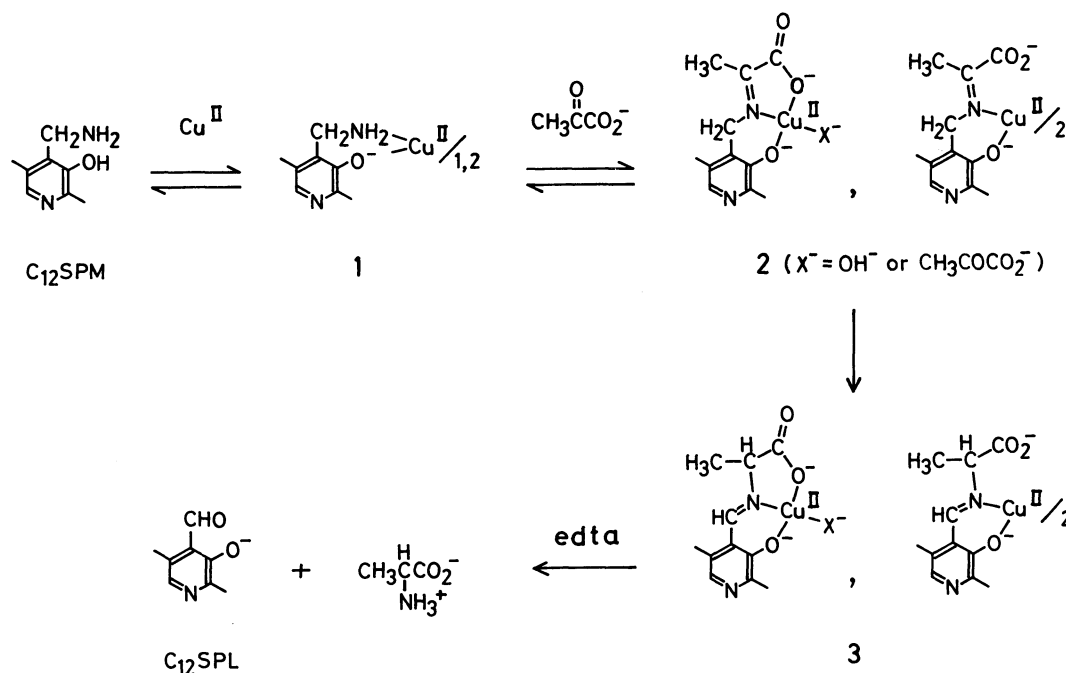


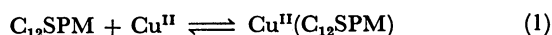
TABLE 1. SPECTRAL PARAMETERS^{a)} FOR CHEMICAL SPECIES INVOLVED IN THE Cu^{II}-CATALYZED TRANSAMINATION OF C₁₂SPM WITH SODIUM PYRUVATE IN VARIOUS AMPHIPHILE SYSTEMS AT 30.0±0.1 °C^{b)}

Species ^{c)}	Amphiphile		
	N ⁺ C ₅ Ala2C ₁₄	N ⁺ C ₅ His2C ₁₄	CTAB
C ₁₂ SPM	293 (4.2×10 ³) 315sh (3.1×10 ³)	294 (4.2×10 ³) 315sh (3.1×10 ³)	295 (3.9×10 ³) 314sh (3.7×10 ³)
Cu ^{II} -C ₁₂ SPM (1)	306	309	305
Cu ^{II} -KSB (2)	305	307	304
Cu ^{II} -ASB (3)	385	392	388

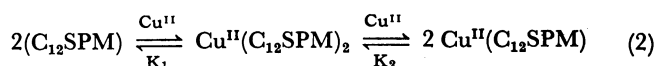
a) Wavelengths (nm) at absorption maxima. Molar absorption coefficients are given in parentheses.
 b) Cationic amphiphile systems in an aqueous HEPES buffer (2.5×10⁻³ mol dm⁻³) at pH 6.8 and μ 0.10 (KCl): N⁺C₅Ala2C₁₄, 1.0×10⁻³ mol dm⁻³; N⁺C₅His2C₁₄, 1.0×10⁻³ mol dm⁻³; CTAB, 3.0×10⁻³ mol dm⁻³. Initial concentrations of reactants: C₁₂SPM, 5.0×10⁻⁵ mol dm⁻³; Cu(ClO₄)₂, 5.0×10⁻⁵ mol dm⁻³; sodium pyruvate, 5.0×10⁻³ mol dm⁻³. c) For structures of metal complexes, see Scheme 2; KSB, ketimine Schiff-base; ASB, aldimine Schiff-base.

α -keto acids and copper(II) ion yielded the Cu^{II}-aldimine chelates in methanol, but the rate-determining step was not unambiguously defined.¹¹⁾ In the present transamination reaction, however, the isomerization of the Cu^{II}-ketimine chelate to the Cu^{II}-aldimine chelate is exclusively the rate-determining step without any ambiguity, since the reaction time course was not influenced by the mixing mode of the reactants, simultaneous or sequential, and showed a monophasic kinetic feature. The absorption bands characteristic of the Cu^{II}-ketimine and -aldimine chelates were used to determine the rates of isomerization reaction.

Metal-coordination Behavior of C₁₂SPM. Prior to the transamination reaction, the copper(II) ion undergoes coordination with C₁₂SPM as shown in Scheme 2. The π - π^* transition bands appearing in the 300-nm range are due to the pyridoxamine chromophore of C₁₂SPM in the N⁺C₅Ala2C₁₄ vesicle, and their intensities increased upon addition of Cu(ClO₄)₂ (Fig. 2). The simplest coordination equilibrium would involve the formation of the 1:1 (C₁₂SPM:Cu^{II}) complex (Eq. 1).



This is not the case, however, since the value of $[\text{Cu}^{\text{II}}(\text{C}_{12}\text{SPM})]/[\text{C}_{12}\text{SPM}][\text{Cu}^{\text{II}}]$ varied as the concentration of Cu(ClO₄)₂ was changed. Alternatively, the copper-coordination scheme, which involves the formation of both 2:1 and 1:1 (C₁₂SPM:Cu^{II}) complexes (Eq. 2), was utilized to analyze the equilibrium data.



The formation of such 2:1 and 1:1 chelate species has been also reported for the coordination of Cu^{II} ion with pyridoxamine in aqueous media.¹²⁾ The observation of an isosbestic point at 286 nm (Fig. 2) assures that the present 2:1 and 1:1 chelates have an identical molar absorption coefficient as given in terms of the C₁₂SPM unit. Now, the respective stability constants (K_1 and K_2), defined by Eqs. 3 and 4, were evaluated by the least-squares analysis in a manner similar to that applied to the zinc(II)-paracyclophane system.¹³⁾

$$K_1 = \frac{[\text{Cu}^{\text{II}}(\text{C}_{12}\text{SPM})_2]}{[\text{C}_{12}\text{SPM}]^2[\text{Cu}^{\text{II}}]} \quad (3)$$

$$K_2 = \frac{[\text{Cu}^{\text{II}}(\text{C}_{12}\text{SPM})]^2}{[\text{Cu}^{\text{II}}(\text{C}_{12}\text{SPM})_2][\text{Cu}^{\text{II}}]} \quad (4)$$

The total concentration of C₁₂SPM bound to Cu^{II} at a given concentration of Cu(ClO₄)₂ ($c = [\text{Cu}^{\text{II}}(\text{C}_{12}\text{SPM})] + 2[\text{Cu}^{\text{II}}(\text{C}_{12}\text{SPM})_2]$) is calculated by Eq. 5.

$$c = \frac{\Delta A}{A_c - A_f} [\text{C}_{12}\text{SPM}]_0 \quad (5)$$

Here, A_c and A_f denote the absorbance in the presence of sufficiently excess Cu(ClO₄)₂ and that in its absence, respectively, at a selected wavelength close to the absorption maxima of the Cu^{II}-C₁₂SPM complexes; ΔA is the absorbance change with respect to A_f at the intermediate concentration range of Cu(ClO₄)₂; and

$[\text{C}_{12}\text{SPM}]_0$ denotes the total concentration of C₁₂SPM. Combining Eqs. 3, 4, and 5, we obtain Eq. 6.

$$(\begin{matrix} K_1 K_2 \end{matrix})^{1/2} [\text{C}_{12}\text{SPM}] ([\text{Cu}^{\text{II}}]_0 - c) + K_1 [\text{C}_{12}\text{SPM}]^2 (2[\text{Cu}^{\text{II}}]_0 - c) = c \quad (6)$$

Here, $[\text{Cu}^{\text{II}}]_0$ is the initial concentration of Cu(ClO₄)₂. This equation is in the form, $aX + bY = Z$, where a and b are $(K_1 K_2)^{1/2}$ and K_1 , respectively, and X , Y , and Z are experimentally observable. K_1 and K_2 were determined by the least-squares analysis using all the experimental sets of (X, Y, Z) at various concentrations of Cu(ClO₄)₂. The results are summarized in Table 2. The observed total concentration of C₁₂SPM bound to Cu^{II} was computationally reproduced quite satisfactorily by the aid of the K_1 and K_2 values determined as above for the whole concentration range of Cu(ClO₄)₂ employed in the present study (Fig. 3).

Figure 4 shows the calculated molar distributions of the 2:1 and 1:1 chelate species of C₁₂SPM in the three different molecular assemblies as a function of the Cu^{II} concentration. In both N⁺C₅Ala2C₁₄ vesicle and CTAB micelle, the fraction of the 2:1 complex is greater than that of the 1:1 complex in a low concentration range of Cu^{II}, while the fraction of the latter exceeds that of the former at higher Cu^{II} concentrations. Meanwhile, the

TABLE 2. STABILITY CONSTANTS FOR COPPER(II) COMPLEXES OF C₁₂SPM IN VARIOUS AMPHIPHILE SYSTEMS AT 30.0 $\pm$ 0.1 °C^{a)}

	N ⁺ C ₅ Ala2C ₁₄	N ⁺ C ₅ His2C ₁₄	CTAB
$K_1/\text{mol}^{-2} \text{ dm}^6$	3.0×10^{10}	1.8×10^{10}	1.1×10^{11}
K_2	0.26	320	42

a) Cationic amphiphile systems in an aqueous HEPES buffer ($2.5 \times 10^{-3} \text{ mol dm}^{-3}$) at pH 6.8 and μ 0.10 (KCl): N⁺C₅Ala2C₁₄, $1.0 \times 10^{-3} \text{ mol dm}^{-3}$; N⁺C₅His2C₁₄, $1.0 \times 10^{-3} \text{ mol dm}^{-3}$; CTAB, $3.0 \times 10^{-3} \text{ mol dm}^{-3}$.

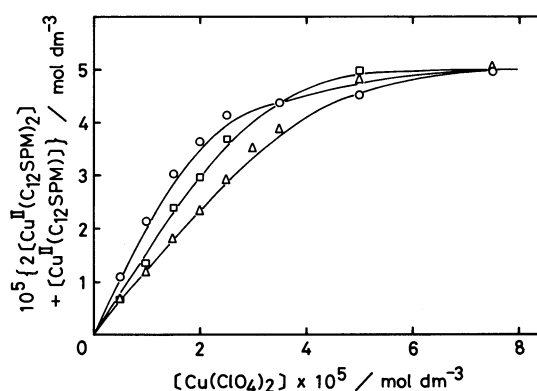


Fig. 3. Correlations between total concentrations of Cu(ClO₄)₂ and Cu^{II}-C₁₂SPM complexes in an aqueous HEPES buffer ($2.5 \times 10^{-3} \text{ mol dm}^{-3}$) at pH 6.8, μ 0.10 (KCl), and 30.0 $\pm$ 0.1 °C in the presence of N⁺C₅Ala2C₁₄ (○, $1.0 \times 10^{-3} \text{ mol dm}^{-3}$), N⁺C₅His2C₁₄ (△, $1.0 \times 10^{-3} \text{ mol dm}^{-3}$), and CTAB (□, $3.0 \times 10^{-3} \text{ mol dm}^{-3}$). Solid lines refer to the calculated data by the aid of evaluated stability constants (K_1 and K_2 , Table 2); total concentration of C₁₂SPM, $5.0 \times 10^{-5} \text{ mol dm}^{-3}$.

1:1 complex is formed predominantly over the whole concentration range of $\text{Cu}(\text{ClO}_4)_2$ in the $\text{N}^+\text{C}_5\text{His}2\text{C}_{14}$ vesicle. This result strongly indicates that the imidazolyl group of $\text{N}^+\text{C}_5\text{His}2\text{C}_{14}$ undergoes coordination with the copper(II) ion which leads to the formation of the 1:1 complex in preference to that of the 2:1 complex.¹⁴⁾

Kinetic Behavior of Isomerization Reaction. The isomerization of the Cu^{II} -ketimine Schiff-base to the corresponding Cu^{II} -aldimine chelate was followed by means of electronic absorption spectroscopy at $30.0 \pm 0.1^\circ\text{C}$ in the presence of cationic amphiphiles.

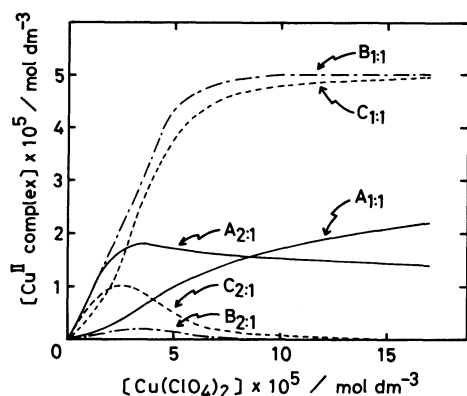


Fig. 4. Distributions of Cu^{II} - C_{12}SPM complexes in an aqueous HEPES buffer ($2.5 \times 10^{-3} \text{ mol dm}^{-3}$) at pH 6.8, μ 0.10 (KCl), and $30.0 \pm 0.1^\circ\text{C}$ in the presence of $\text{N}^+\text{C}_5\text{Ala}2\text{C}_{14}$ (A, $1.0 \times 10^{-3} \text{ mol dm}^{-3}$), $\text{N}^+\text{C}_5\text{His}2\text{C}_{14}$ (B, $1.0 \times 10^{-3} \text{ mol dm}^{-3}$), and CTAB (C, $3.0 \times 10^{-3} \text{ mol dm}^{-3}$). Subscripts 1:1 and 2:1 denote the 1:1 and 2:1 ($\text{C}_{12}\text{SPM}:\text{Cu}^{\text{II}}$) complexes, respectively; total concentration of C_{12}SPM , $5.0 \times 10^{-5} \text{ mol dm}^{-3}$.

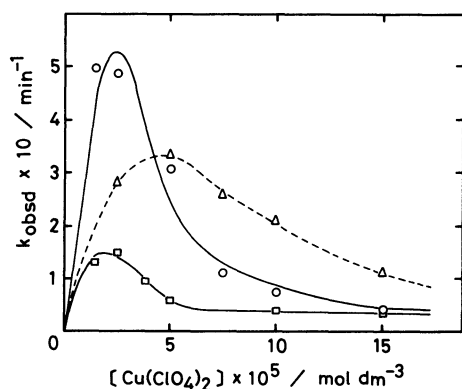
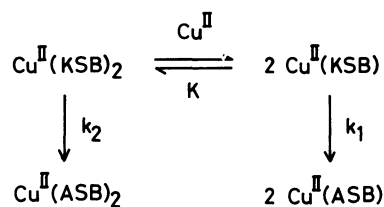


Fig. 5. Correlations between total concentration of $\text{Cu}(\text{ClO}_4)_2$ and first-order rate constant for the isomerization of Cu^{II} -ketimine to Cu^{II} -aldimine in an aqueous HEPES buffer ($2.5 \times 10^{-3} \text{ mol dm}^{-3}$) at pH 6.8, μ 0.10 (KCl), and $30.0 \pm 0.1^\circ\text{C}$ in the presence of $\text{N}^+\text{C}_5\text{Ala}2\text{C}_{14}$ (O, $1.0 \times 10^{-3} \text{ mol dm}^{-3}$), $\text{N}^+\text{C}_5\text{His}2\text{C}_{14}$ (Δ , $1.0 \times 10^{-3} \text{ mol dm}^{-3}$), and CTAB ($\square$, $3.0 \times 10^{-3} \text{ mol dm}^{-3}$) with initial concentrations: C_{12}SPM , $5.0 \times 10^{-5} \text{ mol dm}^{-3}$; sodium pyruvate, $5.0 \times 10^{-3} \text{ mol dm}^{-3}$. Solid lines refer to the calculated data on the basis of Eq. 7 and evaluated kinetic parameters (Table 3).

The absorbance increase due to the formation of the Cu^{II} -aldimine chelate was monitored at 385 nm to determine the reaction rate. The observed rate constant, k_{obsd} , was plotted against the total concentration of $\text{Cu}(\text{ClO}_4)_2$ as shown in Fig. 5.

In both $\text{N}^+\text{C}_5\text{Ala}2\text{C}_{14}$ vesicle and CTAB micelle, the rate constant reaches a maximum and then falls down sharply as the Cu^{II} concentration increases. This overall feature is completely reproducible in several sets of kinetic runs. In the light of the coordination behavior of C_{12}SPM with the copper(II) ion, it can be considered that the copper-binding scheme for the formation of Cu^{II} -ketimine Schiff-base complexes involves both 2:1 and 1:1 (ketimine: Cu^{II}) chelate species as shown in Scheme 2. The observed correlations given in Fig. 5 can be reproduced by summing up the reaction rates of both 1:1 and 2:1 complexes; the former and latter complexes are designated as $\text{Cu}^{\text{II}}(\text{KSB})$ and $\text{Cu}^{\text{II}}(\text{KSB})_2$ with specific rate constants, k_1 and k_2 (per ketimine Schiff-base), respectively. The specific rate constant for the metal-free ketimine Schiff-base is neglected under the present conditions. The kinetic expression, on the basis of Scheme 3, is given by Eq. 7, where $[\text{Cu}^{\text{II}}-\text{KSB}]_{\text{T}}$ is the total concentration of the Cu^{II} -ketimine chelates as given in terms of the ketimine Schiff-base unit.



KSB : ketimine Schiff-base

ASB : aldimine Schiff-base

Scheme 3.

$$k_{\text{obsd}}[\text{Cu}^{\text{II}}-\text{KSB}]_{\text{T}} = k_1[\text{Cu}^{\text{II}}(\text{KSB})] + 2k_2[\text{Cu}^{\text{II}}(\text{KSB})_2] \quad (7)$$

The concentrations of $\text{Cu}^{\text{II}}(\text{KSB})$ and $\text{Cu}^{\text{II}}(\text{KSB})_2$ are interrelated by the stability constant (K) defined by Eq. 8.

$$K = \frac{[\text{Cu}^{\text{II}}(\text{KSB})]^2}{[\text{Cu}^{\text{II}}(\text{KSB})_2][\text{Cu}^{\text{II}}]} \quad (8)$$

The determination of K values was not made by the spectral method which was applied to the Cu^{II} - C_{12}SPM system, because the coordination behavior given by Eq. 8 is accompanied with the isomerization reaction. Accordingly, the values of k_1 , k_2 , and K were obtained by the regression analysis which minimizes the sum of the squares of errors (U) applied to all the experimental data (Eq. 9).

$$U = \sum \{k_{\text{obsd}}[\text{Cu}^{\text{II}}-\text{KSB}]_{\text{T}} - k_1[\text{Cu}^{\text{II}}(\text{KSB})] - 2k_2[\text{Cu}^{\text{II}}(\text{KSB})_2]\}^2 \quad (9)$$

The value of $[\text{Cu}^{\text{II}}-\text{KSB}]_{\text{T}}$ was evaluated from the final concentration of the Cu^{II} -aldimine Schiff-base

TABLE 3. SPECIFIC RATE CONSTANTS FOR ISOMERIZATION REACTION OF Cu^{II}-KETIMINE TO Cu^{II}-ALDIMINE AND STABILITY CONSTANTS FOR Cu^{II}-KETIMINE CHELATES IN AMPHIPHILE SYSTEMS AT 30.0±0.1 °C^{a)}

Parameter	Amphiphile	
	N ⁺ C ₅ Ala2C ₁₄	CTAB
k_1/min^{-1}	0.041	0.036
k_2/min^{-1}	0.70	0.17
K	20	130

a) Cationic amphiphile systems in an aqueous buffer ($2.5 \times 10^{-3} \text{ mol dm}^{-3}$) at pH 6.8 and μ 0.10 (KCl): N⁺C₅Ala2C₁₄, $1.0 \times 10^{-3} \text{ mol dm}^{-3}$; CTAB, $3.0 \times 10^{-3} \text{ mol dm}^{-3}$.

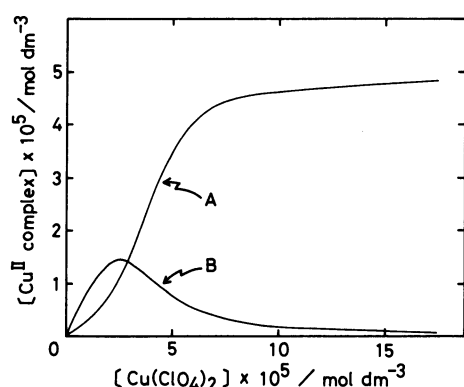


Fig. 6. Distributions of Cu^{II}-ketimine chelates in an aqueous HEPES buffer ($2.5 \times 10^{-3} \text{ mol dm}^{-3}$) at pH 6.8, μ 0.10 (KCl), and $30.0 \pm 0.1^\circ \text{C}$ in the presence of N⁺C₅Ala2C₁₄ vesicle ($1.0 \times 10^{-3} \text{ mol dm}^{-3}$): A, 1:1 (ketimine:Cu^{II}) chelate; B, 2:1 chelate. Initial concentrations: C₁₂SPM, $5.0 \times 10^{-5} \text{ mol dm}^{-3}$; sodium pyruvate, $5.0 \times 10^{-3} \text{ mol dm}^{-3}$.

produced by the isomerization reaction,¹⁵⁾ since the Cu^{II}-ketimine chelate was quantitatively converted into the corresponding Cu^{II}-aldimine chelate.

The kinetic parameters thus obtained for the isomerization carried out in the aggregates of N⁺C₅Ala2C₁₄ and CTAB are listed in Table 3, and the calculated molar distributions of individual chelate species in the N⁺C₅Ala2C₁₄ vesicle are shown in Fig. 6 as a function of the Cu^{II} concentration. In a manner as observed for the Cu^{II}-C₁₂SPM system, the fraction of the 2:1 complex is greater than that of the 1:1 complex in a low Cu^{II} concentration range. However, the formation of the 1:1 complex is more facilitated with the terdentate ketimine Schiff-base than with the bidentate C₁₂SPM. It is noteworthy that the reactivity of the 2:1 complex is much larger than that of the 1:1 complex in the molecular assemblies of N⁺C₅Ala2C₁₄ and CTAB, and that the formation of the former species is more pronounced in the N⁺C₅Ala2C₁₄ vesicle. In order that the isomerization must take place ($2 \rightarrow 3$ in Scheme 2), the hydrogen atom substituted on the carbon atom, interposed between the Schiff-base nitrogen and 4-carbon atom of the pyridine ring, needs to be displaced first. The polarization of the C₄'-H bond of a given Schiff-base chelate is important in facilitating its cleavage. Thus, the effective charge on

Cu^{II} must exert much effect on the reactivity. The 2:1 complex, in which the carboxylate group of the ketimine Schiff-base is free from coordination, retains positive charge on Cu^{II} more effectively than the 1:1 complex, in which three coordination sites are occupied with the anionic groups. Such a charge effect provided on Cu^{II} has been also claimed for the β -elimination reaction of O-phosphoserine with glyoxylate and copper(II) ions.¹⁶⁾

As shown in Fig. 5, the correlation between rate and Cu^{II} concentration observed in the N⁺C₅His2C₁₄ vesicle is considerably different from those in the molecular aggregates of N⁺C₅Ala2C₁₄ and CTAB. In the light of the coordination behavior of C₁₂SPM with the copper(II) ion, the primary species in a concentration range of Cu(ClO₄)₂ higher than $5.0 \times 10^{-5} \text{ mol dm}^{-3}$ must be the 1:1 Cu^{II}-ketimine chelate. However, the observed rate constant reaches a maximum at the Cu^{II} concentration of $5.0 \times 10^{-5} \text{ mol dm}^{-3}$ and then falls down gradually as the Cu^{II} concentration increases beyond this range. This rate profile can be understood on the basis of the coordination behavior of the imidazolyl group of N⁺C₅His2C₁₄ with the copper(II) ion. The coordination interaction of the imidazolyl group with Cu^{II} tends to stabilize the 1:1 Cu^{II}-ketimine chelate since the residual coordination site of Cu^{II} in the 1:1 chelate is occupied with the imidazolyl group and to inhibit the formation of the corresponding 2:1 chelate. In a concentration range of Cu(ClO₄)₂ lower than $5.0 \times 10^{-5} \text{ mol dm}^{-3}$ where all the copper(II) ions are exclusively coordinated to the ketimine Schiff-base, the isomerization reaction of the Cu^{II}-ketimine chelate is catalyzed by the imidazolyl group free from coordination with the copper(II) ion (Fig. 7). We have already confirmed the catalytic assistance of the imidazolyl group as a general base in the transamination reaction of PLP with N-dodecyl-L-alaninamide in the N⁺C₅His2C₁₄ vesicle.⁶⁾ At higher concentrations of Cu(ClO₄)₂ beyond $5.0 \times 10^{-5} \text{ mol dm}^{-3}$, the excess copper(II) ion tends to form the Cu^{II}-imidazole complexes to reduce an amount of the free imidazolyl group. On this ground, the reactivity of the

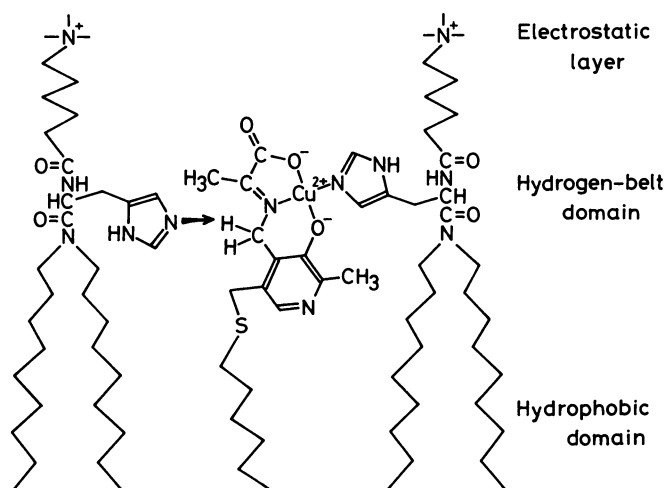


Fig. 7. Schematic representation of the isomerization mechanism for the Cu^{II}-ketimine complex in the N⁺C₅His2C₁₄ vesicle.

1:1 complex in the $N^+C_5\text{His}2C_{14}$ vesicle must be facilitated by the general base catalysis of the metal-free imidazolyl group.

In conclusion, it became apparent that the copper(II)-catalyzed transamination of $C_{12}\text{SPM}$ with pyruvate in the cationic vesicles proceeds to give $C_{12}\text{SPL}$ and alanine as the final products. The detailed kinetic investigation of the isomerization of the Cu^{II} -ketimine to the corresponding Cu^{II} -aldimine clarified the following aspects. (i) In the bilayer vesicle formed with $N^+C_5\text{Ala}2C_{14}$, the formation of the reactive 2:1 (ketimine: Cu^{II}) complex is more favored than in the ordinary cationic micelles. (ii) The bilayer vesicle formed with the amphiphile having the histidyl residue ($N^+C_5\text{His}2C_{14}$) allows the formation of the 1:1 complex in preference to that of the 2:1 complex, and the coordination-free imidazolyl group of the amphiphile effectively catalyzes the isomerization as a general base.

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- 14) An absorption band appeared in the 620-nm range upon addition of $\text{Cu}(\text{ClO}_4)_2$ to the $N^+C_5\text{His}2C_{14}$ vesicle. This is attributable to the ligand-field band due to the coordinated copper(II) ion. However, quantitative evaluation of the stability constant for the $\text{Cu}^{\text{II}}-N^+C_5\text{His}2C_{14}$ complex was not possible due to insolubility of the complex in aqueous media.
- 15) Both 2:1 and 1:1 chelate species were assumed to have an identical molar absorption coefficient per aldimine Schiff-base unit in a similar manner as considered for the $\text{Cu}^{\text{II}}-C_{12}\text{SPM}$ complexes.
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