Copper(II)-Catalyzed Transamination between Pyruvate and Hydrophobic Pyridoxamine Embedded in Synthetic Bilayer Membranes†

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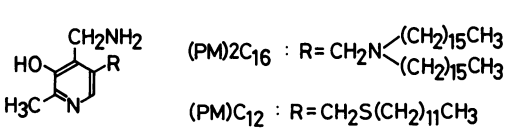
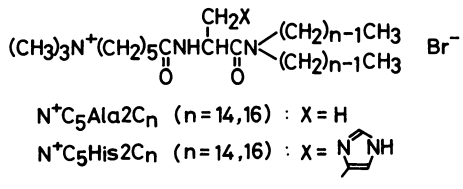
The copper(II)-catalyzed transamination of 4-aminomethyl-5-[(dihexadecylamino)methyl]-2-methyl-3-pyridinol [(PM)2C_{16}] with sodium pyruvate was investigated in an aqueous medium at pH 6.9, μ 0.10 (KCl), and 30.0±0.1 °C in the presence of single-walled bilayer vesicles of *N,N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)-hexanoyl]-L-alaninamide bromide [N^+C_5Ala2C_{16}] and *N,N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide [N^+C_5His2C_{16}]. The binding mode of the pyridoxamine moiety in the vesicles was clarified by comparing the reaction behavior of the (PM)2C_{16}–pyruvate-Cu^{II} system with that of the 4-aminomethyl-5-dodecylthiomethyl-2-methyl-3-pyridinol [(PM)C_{12}]-pyruvate-Cu^{II} system. The pyridoxamine moiety of (PM)2C_{16} was more tightly anchored in the hydrogen-belt domain of the membranes than that of (PM)C_{12} as confirmed by fluorescence polarization spectroscopy. The difference in binding mode between (PM)2C_{16} and (PM)C_{12} is reflected on the copper(II)-coordination equilibria for the formation of the copper(II)-ketimine intermediates. The copper(II)-catalyzed transamination of (PM)2C_{16} with pyruvate in the N^+C_5His2C_{16} vesicle was most enhanced among related reaction systems treated here. The rate enhancement was attributed to the favorable formation of the reactive 2:1 ketimine-copper(II) chelate and the general-base catalysis by the coordination-free imidazolyl group of the lipid.

Bilayer membranes have been utilized as effective media for various organic reactions, exhibiting marked functional assistance.[1–5] The interior hydrophobic domain of bilayer membranes may provide a microenvironment favorable for such reactions due to the following effects: (i) desolvation of reacting species to a significant extent relative to those in homogeneous media; (ii) proximal alignment of reacting species, which is forced by the hydrophobic interactions with organized lipid molecules; (iii) catalytic assistance by some functional groups substituted on lipid molecules. In this regard, the functional simulation of a holoenzyme system composed of an apoprotein and a relevant coenzyme is expected to be made by utilizing a combination of a synthetic bilayer membrane and the corresponding coenzyme factor modified with some hydrophobic substituents.

We have previously reported that the single-walled vesicles composed of synthetic peptide lipids, *N,N*-ditetradecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide [N^+C_5Ala2C_{14}] and *N,N*-ditetradecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide [N^+C_5His2C_{14}], provide reaction environments favorable for functional simulation of the vitamin B_6-dependent transaminase.[6,7] For the transamination of a hydrophobic pyridoxamine derivative, (PM)C_{12}, with pyruvate in the molecular aggregates, the copper(II) ion effectively catalyzed the isomerization of the ketimine Schiff-base to the corresponding aldimine Schiff-base, and the subsequent addition of ethylenediaminetetraacetate afforded the corresponding pyridoxal analogue and alanine as the final products. The kinetic analysis has shown the following characteristic features. (i) The 2:1 and 1:1 ketimine–copper(II) chelates are present during the reaction, and the reactivity of the former chelate is much larger than that of the latter in the N^+C_5Ala2C_{14} vesicle. (ii) The N^+C_5His2C_{14} vesicle allowed the formation of the 1:1 chelate in preference to that of the 2:1 chelate, and the coordination-free imidazolyl group of the lipid molecule effectively catalyzes the isomerization as a general base.

In order to improve the catalytic function of such an artificial transaminase, we prepared a pyridoxamine derivative bearing a hydrophobic double-chain, (PM)-2C_{16}, which is able to interact with matrix membranes more tightly than (PM)C_{12} having only a single hydrophobic chain.[8] We report in this article the reaction behavior of (PM)2C_{16} with pyruvate in the individual vesicles of N^+C_5Ala2C_{16} and N^+C_5His2C_{16} as effected by the copper(II) ion. A kinetic feature observed by anchoring the pyridoxamine moiety in the so-called hydrogen-belt domain[9] of the vesicles is to be discussed in terms of the proximity and orientation effects.





Experimental

General Analyses and Measurements. Elemental analyses were performed at the Microanalysis Center of Kyushu University. ^{1}H-NMR spectra were taken on a Hitachi R-24B spectrometer. A Beckman Φ 71 pH meter equipped with a Beckman 39505 combined electrode was used for pH-measurements after calibration with a combination of appropriate standard aqueous buffers. Electronic absorption spectra were taken on a Union Giken SM-401 high-sensitivity spec-

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trophotometer. Fluorescence polarization measurements were made on a Union Giken FS-501A spectrophotometer equipped with a Sord M200 Mark II microcomputer. The steady-state fluorescence anisotropy (r_s) originated from the pyridoxamine moiety of (PM)2C_{16} or (PM)C_{12} was calculated by Eq. 1, where I is the fluorescence intensity, and the subscripts v and h refer to the orientations, vertical and horizontal, respectively, for the excitation and analyzer polarizers in this sequence. C_f is the grating correction factor, given by I_{hv}/I_{hh}.

$$r_s = (I_{vv} - C_f I_{vh})/(I_{vv} + 2C_f I_{vh}) \quad (1)$$

Materials. Preparation and characterization of hydrophobic pyridoxamines, (PM)C_{12}[7] and (PM)2C_{16},[8] and a peptide lipid having the L-alanine residue, N^+C_5Ala2C_{16},[10] have been described elsewhere. A peptide lipid having the L-histidine residue, N^+C_5His2C_{16}, was prepared by the method adopted for the synthesis of N^+C_5His2C_{14}:[6] a colorless glassy solid, $[\alpha]_D^{20}$ −9.5° (*c* 0.84, EtOH); ^{1}H-NMR ($CDCl_3$, Me_4Si) δ= 0.85 [6H, t, $(CH_2)_{15}\underline{CH_3}$], 1.23 [56H, m, $CH_2(\underline{CH_2})_{14}CH_3$], 1.90 [6H, m, $N^+CH_2(\underline{CH_2})_3CH_2CO$], 2.20 [2H, br t, $N^+(CH_2)_4\underline{CH_2}CO$], 2.80—3.50 [8H, m, N^+CH_2, NCH_2, and $CH\underline{CH_2}Im$], 3.30 [9H, s, $(CH_3)_3N^+$], 5.00 [1H, m, $NH\underline{CH}CO$], 6.91 [1H, s, Im-5H], 7.60 [1H, d, NH], 7.95 [1H, s, Im-2H].

Found: C, 66.60; H, 10.85; N, 8.03%. Calcd for $C_{47}H_{92}BrN_5O_2\cdot(1/2)H_2O$: C, 66.56; H, 11.05; N, 8.26%.

Kinetic Measurements. The copper(II)-catalyzed transamination reaction of (PM)2C_{16} with pyruvate in the single-walled vesicles was monitored spectrophotometrically in an aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonate (HEPES) buffer (2.5×10^{-3} mol dm^{-3}, pH 6.9) containing 0.10 mol dm^{-3} potassium chloride at 30.0±0.1 °C. The experimental details are analogous to those reported previously for the copper(II)-catalyzed reaction of (PM)C_{12} with pyruvate in the individual vesicles of N^+C_5Ala2C_{14} and N^+C_5His2C_{14}.[7]

Results and Discussion

Microenvironments Around the Pyridoxamine Moiety in Vesicles. The peptide lipids, N^+C_5Ala2C_{16} and N^+C_5His2C_{16}, form single-walled vesicles in aqueous media upon sonication as confirmed by electron microscopy.[10] The amino acid residues of the lipids are placed in the so-called hydrogen-belt domain[9] interposed between the hydrophobic region formed with aliphatic double chains and the hydrophilic zone with ionic head groups. In order that an effective catalytic assistance is performed by the imidazolyl group of N^+C_5His2C_{16} in the transamination, it is desirable that the pyridoxamine moiety is anchored in the hydrogen-belt domain so tightly as to interact with the imidazolyl group. The microenvironmental polarity around the pyridoxamine moiety of (PM)C_{12} incorporated into the individual vesicles of N^+C_5Ala2C_{14} and N^+C_5His2C_{14} was evaluated from its electronic absorption spectra, and found to be equivalent to that provided by dioxane–water (1:1 v/v); Kosower's *Z*-value,[11] 87.[7] This polarity value is in good agreement with that for the hydrogen-belt domain of the vesicle composed of *N*,*N*-dihexadecyl-N^{α}-[1-(6-trimethylammoniohexyl)-1,4-dihydro-3-pyridylcarbonyl]-L-alaninamide bromide (*Z*-value, 89);[3] evaluated with attention to the 1,4-dihydronicotinamide moiety which is inevitably fixed in the vicinity of the domain. The pyridoxamine moiety of (PM)2C_{16} is also placed in the similar microenvironment within the vesicles of N^+C_5Ala2C_{16} (*Z*-value, 87).[8]

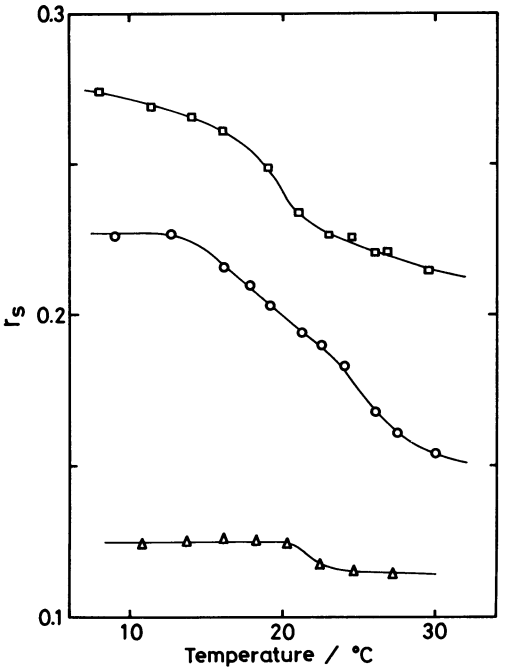
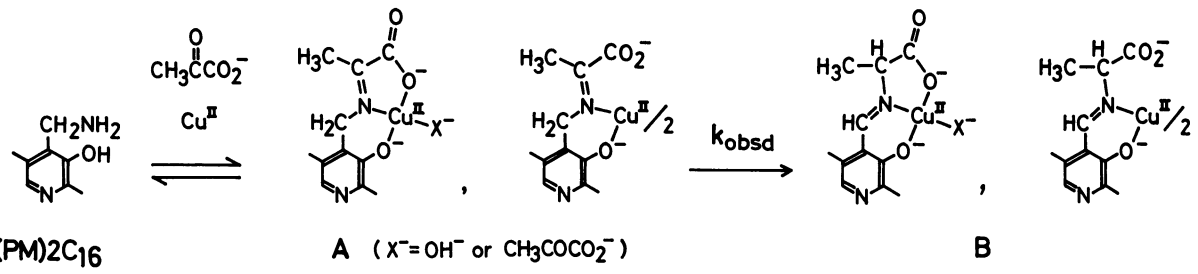


Fig. 1. Correlations between temperature and steady-state fluorescence anisotropy (r_s) for the pyridoxamine moiety (5.0×10^{-5} mol dm^{-3}) in an aqueous HEPES buffer (2.5×10^{-3} mol dm^{-3}) at pH 6.9 and μ 0.10 (KCl) in the presence of single-walled vesicles of peptide lipids (1.0×10^{-3} mol dm^{-3}): ○, (PM)2C_{16} in N^+C_5Ala2C_{16} (taken from Ref. 8); □, (PM)2C_{16} in N^+C_5His2C_{16}; △, (PM)C_{12} in N^+C_5Ala2C_{16}. Excitation and emission wavelengths: 360 and 450 nm for (PM)2C_{16}; 335 and 390 nm for (PM)C_{12}, respectively.

The parameters for steady-state fluorescence anisotropy (r_s) originated from the pyridoxamine moieties of (PM)2C_{16} and (PM)C_{12} in the vesicles were much different from each other. As shown in Fig. 1, the r_s values for (PM)2C_{16} in the individual vesicles of N^+C_5Ala2C_{16} and N^+C_5His2C_{16} are large relative to the corresponding values for (PM)C_{12} in the N^+C_5Ala2C_{16} vesicle over the whole temperature range studied here. The single-walled N^+C_5Ala2C_{16} vesicle shows a broad phase transition range (20±5 °C) as confirmed by differential scanning calorimetry[10] as well as by fluorescence polarization spectroscopy with 1,6-diphenyl-1,3,5-hexatriene embedded in the vesicle.[8] The r_s values for (PM)2C_{16} in the individual vesicles of N^+C_5Ala2C_{16} and N^+C_5His2C_{16} are more sensitive to the phase transition than that for (PM)C_{12} in the N^+C_5Ala2C_{16} vesicle. The result strongly suggests that the pyridoxamine moiety of (PM)2C_{16} is tightly anchored in the hydrogen-belt domain in the vesicles through the hydrophobic interaction of its double-chain portion with the hydrophobic domain of the vesicles. On the other



Scheme 1.

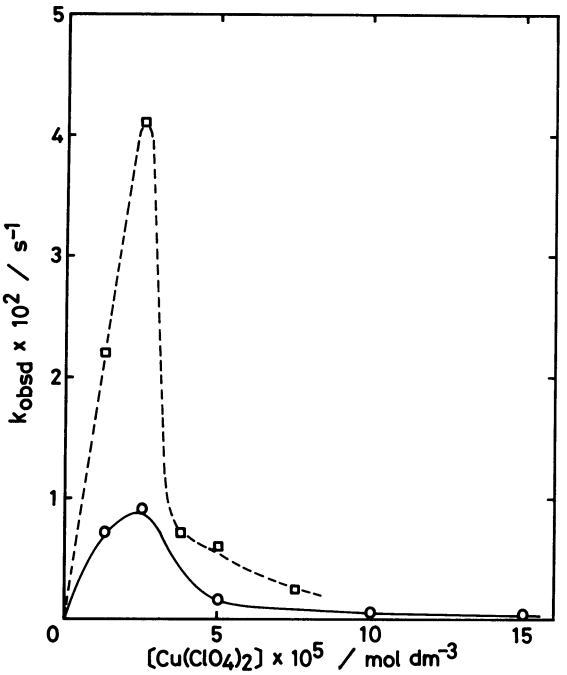
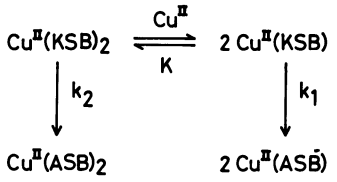


Fig. 2. Correlations between total concentration of $Cu(ClO_4)_2$ and first-order rate constant for the isomerization of Cu^{II}–ketimine to Cu^{II}–aldimine in an aqueous HEPES buffer (2.5×10^{-3} mol dm^{-3}) at pH 6.9, μ 0.10 (KCl), and 30.0±0.1 °C in the presence of $N^+C_5Ala2C_{16}$ (○, 1.0×10^{-3} mol dm^{-3}) and $N^+C_5His2C_{16}$ (□, 1.0×10^{-3} mol dm^{-3}) with initial concentrations: $(PM)2C_{16}$, 5.0×10^{-5} mol dm^{-3}; sodium pyruvate, 5.0×10^{-3} mol dm^{-3}. A solid line refers to the calculated data on the basis of Scheme 2 and evaluated kinetic parameters (Table 1).

hand, the pyridoxamine moiety of $(PM)C_{12}$ is more mobile in the vesicles than that of $(PM)2C_{16}$, although the equilibrium locations of the pyridoxamine moieties of both pyridoxamine derivatives are nearly identical. In addition, the larger r_s values for $(PM)2C_{16}$ in the $N^+C_5His2C_{16}$ vesicle relative to the corresponding values in the $N^+C_5Ala2C_{16}$ vesicle over the whole temperature range may reflect the associative interactions, hydrogen-bonding and π-π interactions, between the pyridoxamine moiety and the imidazolyl group of the former lipid. Such interactions result in repression of the molecular motion of $(PM)2C_{16}$.

Transamination Reaction in $N^+C_5Ala2C_n$ Vesicles. The reaction was initiated by adding copper(II) perchlorate and sodium pyruvate to the covesicles of $N^+C_5Ala2C_{16}$ and $(PM)2C_{16}$, and the progress of the transamination was monitored by spectrophotometric



KSB: ketimine Schiff-base
ASB: aldimine Schiff-base

Scheme 2.

means. In a manner as observed for the copper(II)-catalyzed transamination of $(PM)C_{12}$ with pyruvate in the $N^+C_5Ala2C_{14}$ vesicle,[7] the reaction proceeded through the fast equilibrated formation of the copper(II) complexes of ketimine Schiff-base (**A** in Scheme 1), followed by much slower conversion into the copper(II) complexes of aldimine Schiff-base (**B** in Scheme 1). The isomerization was followed by monitoring an absorption increase at 390 nm, attributable to the formation of the copper(II)–aldimine chelates, and consistent with the first-order kinetics for each run. The observed rate constant, k_{obsd}, was plotted against the total concentration of $Cu(ClO_4)_2$ as shown in Fig. 2. The rate constant reaches a maximum and then falls down as the copper(II) concentration further increases. This kinetic feature is similar to that observed for the copper(II)-catalyzed reaction of $(PM)C_{12}$ with pyruvate in the $N^+C_5Ala2C_{14}$ vesicle.[7] The kinetic analysis was performed on the basis of Scheme 2 in a manner as reported previously[7] under conditions that both of the 2:1 and 1:1 (ketimine:Cu^{II}) chelate species are present. The kinetic parameters thus obtained for the isomerization are listed in Table 1, and the calculated molar distributions of the individual chelate species are shown in Fig. 3 against the copper(II) concentration. The reactivity of the 2:1 chelate is much larger than that of the 1:1 chelate in each reaction system, and the effective charge on the copper(II) atom seems to exert much effect on the reactivity as discussed previously.[7]

The formation of all the copper(II)-ketimine species, evaluated from the amounts of the copper(II)-aldimine chelates produced by the isomerization, is more pronounced in the $(PM)2C_{16}$–pyruvate–Cu^{II} system than in the $(PM)C_{12}$–pyruvate–Cu^{II} system: 88 and 78% mole fractions of the pyridoxamine moiety are transformed into the copper(II)-ketimine chelates for the former and the latter, respectively, at 2.5×10^{-5}

Table 1. Specific rate constants for isomerization reaction of Cu^{II}-ketimine to Cu^{II}-aldimine and stability constants for Cu^{II}-ketimine chelates in $N^+C_5Ala2C_n$ (1.0×10^{-3} mol dm^{-3}) vesicles at 30.0 ± 0.1 °C[a)]

Parameter	(PM)2C16 in $N^+C_5Ala2C_{16}$[b)]	(PM)C12 in $N^+C_5Ala2C_{14}$[c)]
k_1/s^{-1}	2.7×10^{-4}	6.8×10^{-4}
k_2/s^{-1}	1.0×10^{-2}	1.2×10^{-2}
K	170	20

a) In an aqueous HEPES buffer (2.5×10^{-3} mol dm^{-3}) at pH 6.9 and μ 0.10 (KCl). b) Initial concentrations in mol dm^{-3}: (PM)2C16, 5.0×10^{-5}; sodium pyruvate, 5.0×10^{-3}; $Cu(ClO_4)_2$, 1.3×10^{-5}—1.5×10^{-4}. c) Ref. 7.

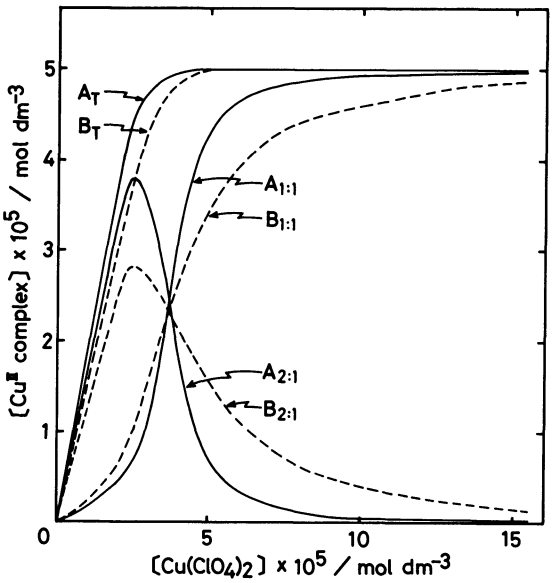


Fig. 3. Distributions of Cu^{II}-ketimine chelates in an aqueous HEPES buffer (2.5×10^{-3} mol dm^{-3}) at pH 6.9, μ 0.10 (KCl), and 30.0 ± 0.1 °C in the presence of $N^+C_5Ala2C_n$ (1.0×10^{-3} mol dm^{-3}): A, (PM)2C16 (5.0×10^{-5} mol dm^{-3})-pyruvate (5.0×10^{-3} mol dm^{-3})-Cu^{II} in $N^+C_5Ala2C_{16}$; B, (PM)C12 (5.0×10^{-5} mol dm^{-3})-pyruvate (5.0×10^{-3} mol dm^{-3})-Cu^{II} in $N^+C_5Ala2C_{14}$ (taken from Ref. 7). Subscripts T, 1:1 and 2:1 denote the total, 1:1 and 2:1 (ketimine:Cu^{II}) chelate species, respectively; [Cu^{II} complex] is given in terms of the Schiff-base unit.

mol dm^{-3} of $Cu(ClO_4)_2$ where the maximum rates were observed for the respective systems (Fig. 3). This clearly indicates that the tight fixation of the pyridoxamine moiety of (PM)2C16 in the hydrogen-belt domain entropically favors the formation of the copper(II)-ketimine chelates and further enhances the formation of the 2:1 ketimine-copper(II) chelate, as compared with the corresponding reaction system involving (PM)C12, in a lower concentration range of the copper(II) ion ([$Cu(ClO_4)_2$]$<3.8\times10^{-5}$ mol dm^{-3}): *i.e.*, molar ratios of the 2:1 chelate to the 1:1 chelate (in terms of the Schiff-base unit) at 2.5×10^{-5} mol dm^{-3} $Cu(ClO_4)_2$ are 6.3 and 2.5 for the (PM)2C16 and (PM)-C12 systems, respectively (Fig. 3).

Comparison of the stability constants for the formation of the 1:1 ketimine-copper(II) complex, as defined by Eq. 2, provides another important information.

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

The K value for the (PM)2C16 system is larger than that for the (PM)C12 system, and this requires some explanation. The efficient hydrophobic interaction of the two double-chain segments of the 2:1 ketimine-copper(II) chelate derived from (PM)2C16 with the hydrophobic vesicle domain acts to anchor the pyridoxamine moieties tightly in the intramembrane region, and consequently mobility of the pyridoxamine moieties are much repressed relative to those of the corresponding complex of (PM)C12. The resulting side-by-side arrangement of the pyridoxamine moieties of (PM)2C16 molecules with the lipid molecules in the hydrogen-belt domain induces a large steric strain within the chelate molecule since the copper(II) ion is forced to take some distorted coordination geometry away from its favorite square-planar one under such circumstances. The strain energy released upon formation of the 1:1 chelate must be greater than that for the corresponding chelate derived from (PM)C12. The steric strain effect is reflected on the activation entropy values observed above the phase transition temperature of $N^+C_5Ala2C_{16}$: for the 2:1 chelate, −2.43; for the 1:1 chelate, +23.3 J K^{-1} mol^{-1}.[8)]

Transamination Reaction in $N^+C_5His2C_n$ Vesicles. The reaction behavior of the copper(II)-catalyzed transamination in the $N^+C_5His2C_n$ vesicles is much influenced by the nature of coenzyme factors. In the (PM)2C16-pyruvate-Cu^{II}-$N^+C_5His2C_{16}$ system, the rate constant reaches a maximum at a 1:2 molar ratio of copper(II) to (PM)2C16 and then falls down sharply as the copper(II) concentration further increases (Fig. 2). The conversions to the aldimine-copper(II) chelate are 50 and 100% on the pyridoxamine base at the 4:1 and 2:1 molar ratios of (PM)2C16 to copper(II), respectively, as confirmed by electronic absorption spectroscopy. This clearly indicates that the copper(II) ion is quantitatively converted into the 2:1 ketimine-copper(II) chelate under such conditions. On this basis, the specific rate constant evaluated for the 2:1 ketimine-copper(II) chelate in the $N^+C_5His2C_{16}$ vesicle, 0.041 s^{-1}, is 4 times larger than that for the same complex in the $N^+C_5Ala2C_{16}$ vesicle, indicating that the catalytic assistance is performed by the imidazolyl group of $N^+C_5His2C_{16}$ as a general base.

In the copper(II) concentration range higher than the 1:2 molar ratio of copper(II) *vs.* (PM)2C16, the reactivity is sharply reduced. This behavior is presumably originated from the two factors: transformation of the 2:1 ketimine-copper(II) chelate into the 1:1 chelate, which has lower reactivity relative to the former; coordination of the imidazolyl group to the copper(II) atom which results in masking of its catalytic activity.

As for the (PM)C12-pyruvate-Cu^{II}-$N^+C_5His2C_{14}$ system, conversion to the aldimine-copper(II) chelate reaches a value of 50% on the pyridoxamine base at

the 2:1 molar ratio of (PM)C_{12} to copper(II), indicating that the 1:1 ketimine–copper(II) chelate is present as the predominant species.[7] Even though the 2:1 chelate may be more reactive than the 1:1 complex in the light of the above observations, the larger fractional distribution of the 1:1 chelate over the whole copper(II) concentration range studied seems to be responsible for the lower reactivity relative to the reaction system involving (PM)2C_{16}.

In conclusion, it became apparent that the binding mode of the pyridoxamine moiety of the coenzyme analogues in the synthetic bilayer membranes appreciably controls the reaction behavior of the copper(II)-catalyzed transamination with pyruvate. The tight anchoring of the pyridoxamine moiety in the hydrogen-belt domain of the vesicles as observed with (PM)-2C_{16} results in the favorable formation of the reactive 2:1 ketimine–copper(II) chelate, and consequently the transamination reaction is much accelerated. The holoenzyme model system composed of (PM)2C_{16}, N^+-C_5His2C_{16}, and the copper(II) ion showed the extremely large reactivity for the transamination with pyruvate, which is originated from the bifunctional assistance by the copper(II) ion and the coordination-free imidazolyl group of N^+C_5His2C_{16}; the former acts to produce the reactive 2:1 chelate while the latter catalyzes the reaction as a general base.

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References

1) M. F. Czarniecki and R. Breslow, *J. Am. Chem. Soc.*, **101**, 3675 (1979).
2) Y. Murakami, A. Nakano, A. Yoshimatsu, and K. Fukuya, *J. Am. Chem. Soc.*, **103**, 728 (1981).
3) Y. Murakami, J. Kikuchi, K. Nishida, and A. Nakano, *Bull. Chem. Soc. Jpn.*, **57**, 1371 (1984).
4) T. Kunitake, H. Ihara, and Y. Okahata, *J. Am. Chem. Soc.*, **105**, 6070 (1983); and references cited therein.
5) R. C. Petter, J. C. Mitchell, W. J. Brittain, T. J. McIntosh, and N. A. Porter, *J. Am. Chem. Soc.*, **105**, 5700 (1983).
6) Y. Murakami, A. Nakano, and K. Akiyoshi, *Bull. Chem. Soc. Jpn.*, **55**, 3004 (1982).
7) Y. Murakami, J. Kikuchi, A. Nakano, K. Akiyoshi, and T. Imori, *Bull. Chem. Soc. Jpn.*, **57**, 1116 (1984).
8) Y. Murakami, J. Kikuchi, and K. Akiyoshi, *Chem. Lett.*, **1984**, 1185.
9) Y. Murakami, A. Nakano, and H. Ikeda, *J. Org. Chem.*, **47**, 2137 (1982).
10) Y. Murakami, A. Nakano, A. Yoshimatsu, K. Uchitomi, and Y. Matsuda, *J. Am. Chem. Soc.*, **106**, 3613 (1984).
11) E. M. Kosower, *J. Am. Chem. Soc.*, **80**, 3253 (1958).