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COPPER(II)-CATALYZED TRANSAMINATION OF HYDROPHOBIC PYRIDOXAMINE WITH PYRUVIC ACID IN BILAYER MEMBRANE: PHASE TRANSITION EFFECT

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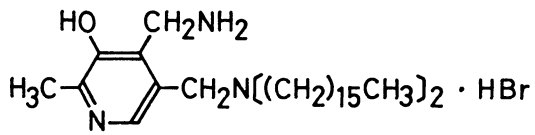
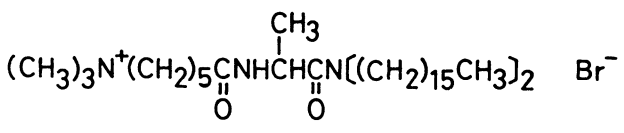
The gel–liquid-crystalline phase transition effect on the copper(II)-catalyzed transamination of 2-methyl-3-hydroxy-4-aminomethyl-5-[(N,N-dihexadecylamino)methyl]pyridine with sodium pyruvate was investigated in single-walled bilayer vesicles of N,N-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide.

Currently, there is growing interest in the utilization of bilayer membranes as organized media for chemical reactions. Since the molecular fluidity of such membranes undergoes drastic changes by phase transition between the gel and liquid-crystalline states, the reactivity and reactant selectivity of reactions occurring in membranes are expected to be manipulated by the phase properties.[1] Furthermore, bilayer membranes can be utilized to simulate the microenvironmental properties and functions of apoenzymes. On the basis of these viewpoints, we composed in this work a holoenzyme model having vitamin B_6 activity with the covesicles of $N^+C_5Ala2C_{16}$[2] and a hydrophobic pyridoxamine analogue, $(PM)2C_{16}$,[3] and investigated the copper(II)-catalyzed transamination of $(PM)2C_{16}$ with pyruvic acid.

An aqueous dispersion of $N^+C_5Ala2C_{16}$ (1.0 mmol dm^{-3}) containing $(PM)2C_{16}$ (5.0×10^{-2} mmol dm^{-3}) in an aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonate (HEPES) buffer (25 mmol dm^{-3}, pH 6.9, μ 0.10 with KCl) was sonicated for 1 min with a probe-type sonicator at 30 W to obtain single-walled vesicles. The aggregate morphology of the present vesicles was intrinsically identical with that of the single-walled vesicles composed of $N^+C_5Ala2C_{16}$ without any coexisting component as confirmed by electron microscopy. The single-walled $N^+C_5Ala2C_{16}$ vesicle was found to show a broad phase transition at 20 ± 5 °C by differential scanning calorimetry (DSC)[2] and fluorescence polarization spectroscopy with 1,6-diphenyl-1,3,5-hexatriene (DPH) embedded in the membrane (Fig. 1). The phase transition temperature for the present functionalized vesicle was evaluated by measuring temperature dependence of steady-state fluorescence anisotropy (r_s)[5] originated from the pyridoxamine moiety of $(PM)2C_{16}$. As shown in Fig. 1, the phase transitions of both



$(PM)2C_{16}$



$N^+C_5Ala2C_{16}$

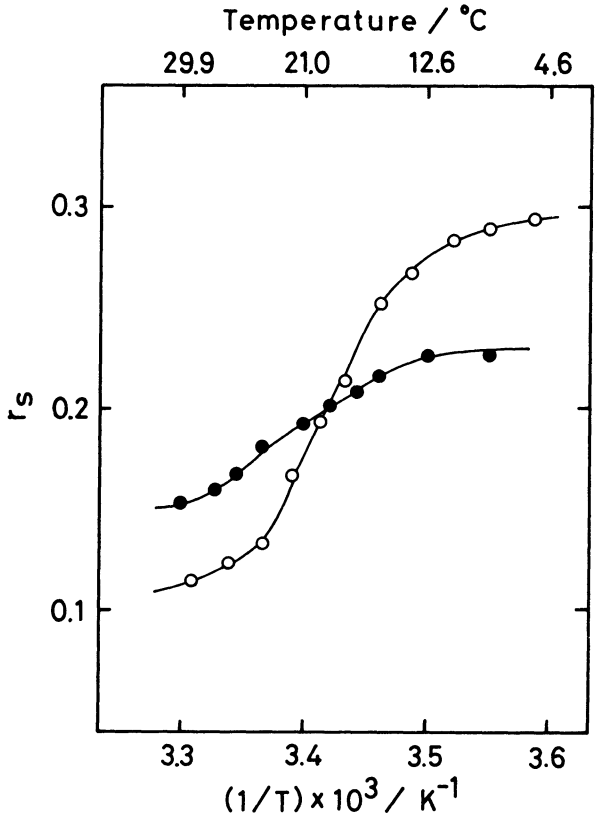
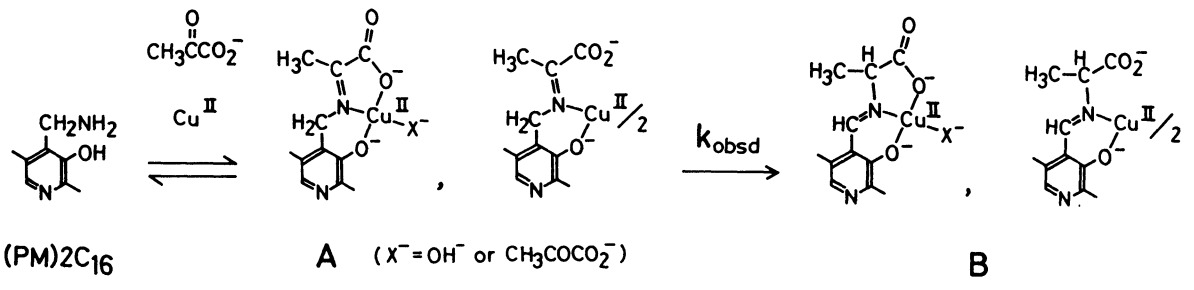


Fig. 1. Correlation between temperature and steady-state fluorescence anisotropy (r_s) in an aqueous HEPES buffer in the presence of single-walled vesicles of 1.0 mmol dm^{-3} $N^+C_5Ala2C_{16}$ with fluorescent probes: DPH (○), 1.0 x 10^{-4} mmol dm^{-3}; (PM)$2C_{16}$ (●), 5.0 x 10^{-2} mmol dm^{-3}. Excitation: DPH, 358 nm; (PM)-$2C_{16}$, 360 nm. Emission at 450 nm for both probes.

vesicles with and without (PM)$2C_{16}$ occur in the same temperature range. The r_s value is more sensitively varied with DPH than with the pyridoxamine moiety as temperature is changed. This must reflect the difference between these fluorescent probes in their incorporated sites; DPH is placed in the hydrophobic domain[6] while the pyridoxamine moiety of (PM)$2C_{16}$ in the so-called hydrogen-belt domain interposed between the hydrophobic interior and the hydrophilic surface region.[7]

The reaction was initiated by adding copper(II) perchlorate (2.5 x 10^{-2} or 0.1 mmol dm^{-3}) and sodium pyruvate (5.0 mmol dm^{-3}) to the covesicles of $N^+C_5Ala2C_{16}$ and (PM)$2C_{16}$, and the progress of the transamination was monitored by spectrophotometric means. In analogy with the copper(II)-catalyzed transamination of 2-methyl-3-hydroxy-4-aminomethyl-5-dodecylthiomethylpyridine, (PM)SC_{12}, with pyruvic acid in vesicles,[8] the reaction proceeded through the fast equilibrated formation of the Cu^{II}-ketimine Schiff-base complex (A in Scheme 1), followed by much slower conversion into the Cu^{II}-aldimine Schiff-base (B in Scheme 1). The isomerization was



Scheme 1.

followed by monitoring an absorbance increase at 390 nm, attributable to the formation of the aldimine chelate, and consistent with the first-order kinetics for each run. An Arrhenius plot for the transamination in the presence of the copper(II) ion at a 1:2 molar ratio of Cu^{II} vs. $(PM)2C_{16}$ (system A) provided two different lines with a break point in the 15-20 °C range, while only a single straight line was drawn over the whole temperature range studied in the presence of a two-fold molar excess of the copper(II) ion over $(PM)2C_{16}$ (system B). The phase transition was found to occur in the 20 °C range for both systems on the basis of correlation of temperature with r_s by using DPH embedded in the vesicles after the reaction was completed.

We have recently clarified that the copper(II)-catalyzed transamination of $(PM)SC_{12}$ with pyruvic acid in vesicles proceeds via formation of the 2:1 and 1:1 (ketimine : Cu^{II}) complexes.[8] Applying the similar analysis to the present $(PM)2C_{16}$–pyruvate–Cu^{II} system, we found that the predominant ketimine species in systems A and B are the 2:1 and the 1:1 complex, respectively: 76 and 12% mole fractions of $(PM)2C_{16}$ are transformed into the 2:1 and the 1:1 complex, respectively, in system A; while $(PM)2C_{16}$ is quantitatively converted into the 1:1 complex in system B. In addition, the metal-coordination equilibria were hardly affected over the whole temperature range employed.[9] The lipid molecules are much rigidly assembled in the gel state so that the double-chain segment of $(PM)2C_{16}$ is forced

Table 1. Kinetic and activation parameters for the copper(II)-catalyzed transamination of $(PM)2C_{16}$ with pyruvate in $N^+C_5Ala2C_{16}$ vesicle[a)]

$[Cu(ClO_4)_2]$ / mol dm^{-3}	Temp / °C	$k_{obsd} \times 10^4$ / s^{-1}	$\Delta G^{\ddagger}$ / kJ mol^{-1}	$\Delta H^{\ddagger}$ / kJ mol^{-1}	$\Delta S^{\ddagger}$ / J K^{-1} mol^{-1}
2.5×10^{-5}	27.0	98.0	85.4 (298 K)	84.5	-2.43
	26.0	81.5			
	22.0	49.8			
	20.0	36.3			
	17.0	20.7			
	14.5	13.6	85.8 (283 K)	56.1	-104
	11.0	11.0			
	8.5	7.87			
	7.5	7.67			
1.0×10^{-4}	29.8	7.22	93.3 (283 K)	99.6	23.3
	24.0	2.75			
	21.5	2.35			
	18.5	1.49			
	16.0	0.89			
	12.0	0.62			
	9.0	0.37			

a) Initial concentrations in mol dm^{-3}: $(PM)2C_{16}$, 5.0×10^{-5}; sodium pyruvate, 5.0×10^{-3}; $N^+C_5Ala2C_{16}$, 1.0×10^{-3}.

to be incorporated tightly into the hydrophobic vesicle domain. Such rigid molecular arrangement must induce a significant steric strain in the 2:1 complex which results in the formation of a distorted square-planar coordination structure around the nuclear copper atom. The steric effect is apparently reflected on the activation entropy and enthalpy values (Table 1). The activation parameters for the reaction of the 2:1 complex above the phase-transition temperature range approach to the corresponding values for the reaction of the 1:1 complex due to the increased mobility of the membrane lipid molecules and the (PM)$2C_{16}$ molecules.

References

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2) Y. Murakami, A. Nakano, A. Yoshimatsu, K. Uchitomi, and Y. Matsuda, J. Am. Chem. Soc., in press.

3) 2-Methyl-3-hydroxy-4-aminomethyl-5-[(N,N-dihexadecylamino)methyl]pyridine hydrobromide, (PM)$2C_{16}$, was prepared by the reaction of the diacetyl derivative of 2-methyl-3-hydroxy-4-aminomethyl-5-bromomethylpyridine dihydrobromide[4] with dihexadecylamine and the subsequent hydrolysis: a yellow oil; ^{1}H-NMR ($CDCl_3$, Me_4-Si) δ 0.89 [6H, t, $(CH_2)_{15}\underline{CH}_3$], 1.28 [56H, m, $CH_2(\underline{CH}_2)_{14}CH_3$], 2.47 [3H, s, 2-$CH_3$], 2.40-2.75 [4H, m, $\underline{CH}_2(CH_2)_{14}CH_3$], 3.80 [2H, s, 5-$CH_2$], 4.28 [2H, s, 4-$CH_2$], and 7.85 [2H, s, 6-H]. Found: C, 68.40; H, 11.41; N, 5.83%. Calcd for $C_{40}H_{78}BrN_3O + \frac{1}{2}H_2O$: C, 68.10; H, 11.22; N, 5.96%.

4) T. Sakuragi and F. A. Kummerow, Arch. Biochem. Biophys., 71, 303 (1957).

5) The r_s value 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}.

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

6) M. Shinitzky and Y. Barenholz, J. Biol. Chem., 249, 2652 (1974); B. R. Lentz, Y. Barenholz, and T. E. Thompson, Biochemistry, 15, 4521 (1976); M. P. Andrich and J. M. Vanderkooi, ibid., 15, 1257 (1976).

7) The microenvironment for the pyridoxamine moiety is equivalent to that provided by dioxane–water (1:1 v/v) in polarity (Kosower's Z-value, 87). As for the Z-values, see: J. Llor and M. Cortijo, J. Chem. Soc., Perkin Trans. 2, 1977, 1111.

8) Y. Murakami, J. Kikuchi, A. Nakano, K. Akiyoshi, and T. Imori, Bull. Chem. Soc. Jpn., 57, 1116 (1984).

9) The 1:1 and the 2:1 ketimine chelate were directly converted into the corresponding aldimine chelates. The relative yields of the aldimine chelates remained unchanged over the whole temperature range, above and below the phase transition temperature. Thus, the phase separation effect, if any, on the metal-coordination equilibria in the gel state can be neglected.

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