Enantioselective Catalysis by a Supramolecular Bilayer Membrane as an Artificial Aminotransferase. Stereochemical Roles of an L-Lysine Residue and L-Phenylalanine at the Reaction Site

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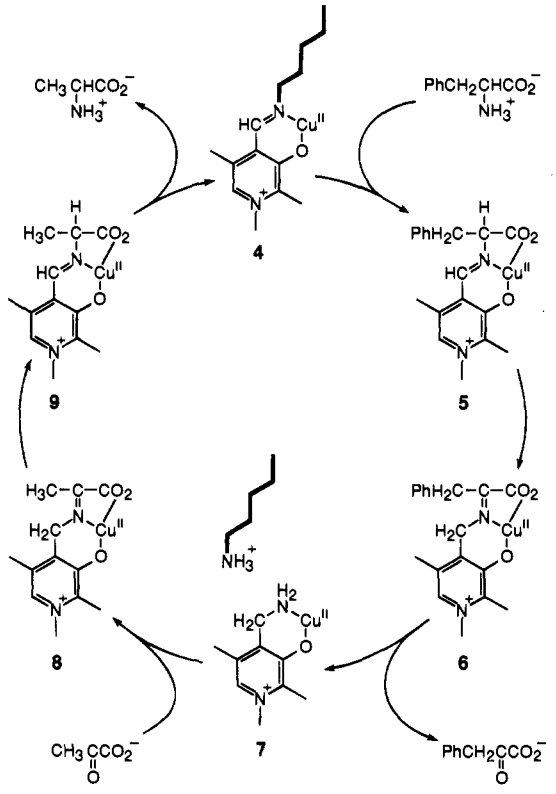
Molecular recognition by functionalized bilayer membranes formed with various molecular species is of great current interest in view of development of artificial supramolecules capable of mimicking chemical functions of enzymes and receptors.[1,2] In particular, the chiral recognition ability of bilayer assemblies and its consequence in stereospecific reactions are essential but insufficiently covered subjects up to the present time.[3] We have recently demonstrated that catalytic functions performed by naturally occurring vitamin B_6-dependent enzymes can be simulated by artificial supramolecules constituted with a bilayer-forming peptide lipid, a hydrophobic vitamin B_6 derivative, and metal ions.[2,4] The resulting functionalized bilayer assemblies exhibited marked substrate selectivities and efficient catalytic activities showing turnover behavior in transamination, β-replacement, and aldolase-type reactions under mild conditions. In the course of our studies on enantioselective catalysis performed by the artificial vitamin B_6-dependent enzymes,[5] we have found that a supramolecular bilayer membrane formed with a peptide lipid having an L-lysine residue (**1a**),[4c] a hydrophobic pyridoxal derivative (**2**),[5a] and copper(II) ions is a markedly efficient artificial aminotransferase showing both high enantioselectivity and turnover behavior. It needs to be pointed out at this stage that α-keto acids afforded the corresponding α-amino acids by the half-transamination reaction mediated by chiral pyridoxamine derivatives as reported previously.[6] However, none of those reactions exhibited turnover behavior of the catalyst species.

Bilayer vesicles were prepared by sonication of an aqueous dispersion containing **1a** and **2** in a 20:1 molar ratio with a probe-type sonicator at 30 W for 30 s. The vesicular size as evaluated by means of dynamic light scattering measurements is 146 nm, and the formation of bilayer vesicles was confirmed by negative-staining electron microscopy. The transamination reaction between phenylalanine (Phe) and pyruvate as mediated by the present bilayer vesicle was carried out in an aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (25 mM, pH 7.0, μ 0.1 with KCl) at 30.0 °C in the presence of copper(II) ions. An electronic absorption spectrum for the resulting enzyme model showed an absorption maximum at 385 nm in the absence of substrates, reflecting quantitative formation of a copper(II) chelate of the internal aldimine Schiff base of **2** with **1a** (**4** in Scheme 1). The enantiomers of alanine (Ala) produced in the course of reaction were treated with *o*-phthalaldehyde in the presence of D-3-mercapto-2-methylpropionic acid to give the corresponding fluorescent isoindole derivatives, diastereomers to each other, and the diastereomers were subsequently separated by reversed-phase HPLC on a column of Inertsil ODS-80A (GL Sciences, Tokyo, Japan).[7]

Scheme 1



The present catalytic bilayer assembly exhibited turnover behavior for the transamination between L-Phe and pyruvate to form D-Ala with high enantiomeric excess (ee) (Table 1, entry 1). This means that the reaction proceeds in accordance with a catalytic cycle shown in Scheme 1.[8] In order to clarify the stereochemical factors affecting the enantioselectivity in the bilayer membrane, we carried out the transamination reaction of L- or D-Phe with pyruvate by utilizing various combinations of supramolecular elements (Chart 1). The results (Table 1) show that the highest enantioselectivity observed for the **1a**−**2**−Cu(II) system comes from the following supramolecular assistance. (1) In these bilayer systems, Phe as one of the substrates is responsible for inducing the opposite chirality in Ala. (2) The protonated ε-amino group of the L-lysine residue involved in **1a** enhanced the formation of D-Ala much more favorably than that of L-Ala (Table 1, entries 1 and 2),[9] while such a difference in the ee value was not observed with **1b**

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(8) We have previously clarified that the transamination reaction in the bilayer vesicular system proceeds via formation of **5**−**9** as shown in Scheme 1; see ref 2 and references cited therein.

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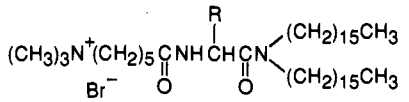
Table 1. Yields and Enantioselectivity Values for Transamination of Phe with Pyruvate Catalyzed by Artificial Aminotransferases[a]

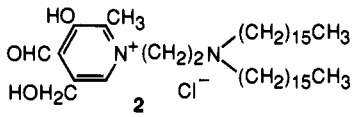
entry	catalyst	chirality of Phe	time (h)	Ala yield (%)	Ala chirality	Ala ee (%)
1	**1a**−**2**−Cu(II)	L	24	68	D	90
			48	108	D	92
			72	135	D	92
			96	152	D	87
			120	167	D	84
2	**1a**−**2**−Cu(II)	D	24	113	L	29
			48	186	L	27
			72	235	L	26
			96	266	L	26
			120	294	L	25
3	**1b**−**2**−Cu(II)	L	24	21	D	21
4	**1b**−**2**−Cu(II)	D	24	24	L	20
5	**1a**−**3a**−Cu(II)	L	24	55	D	53
6	**1a**−**3a**−Cu(II)	D	24	59	L	34
7	**1a**−**3b**−Cu(II)	*b*	1	78	DL	0

[a] In an aqueous HEPES buffer (25 mM, pH 7.0, μ 0.1 with KCl) at 30.0 °C. Concentrations in mM: Phe, 5.0; pyruvate, 5.0; **1**, 1.0; **2** or **3**, 0.050; $Cu(ClO_4)_2$, 0.050. Yields of Ala are based on the amount of a hydrophobic vitamin B_6 analogue. All yield and ee values observed under the present reaction conditions were well reproducible and are accurate within ±3%. [b] In the absence of Phe.

Chart 1



1a R = $(CH_2)_4NH_2$
1b R = CH_3



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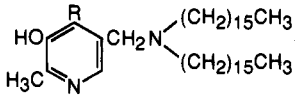

3a R = CHO
3b R = CH_2NH_2

having an L-alanine residue (Table 1, entries 3 and 4). (3) Addition of dioxane to the HEPES buffer medium [HEPES buffer−dioxane (15:85 v/v)] destroyed orderly arrangement of assembly species, and the enantioselectivity completely disappeared as a consequence.[10] (4) A difference in the ee value observed for Ala between the **1a**−**2**−Cu(II) and **1a**−**3a**−Cu(II) systems implies that the stereochemical control is sensitive to the orientation of the pyridoxal moiety in the bilayer membrane (Table 1, entries 5 and 6). (5) The presence of a chiral Phe is indispensable to perform the enantioselective transamination to give a chiral Ala in the bilayer membrane, since Ala produced in the half-transamination of pyruvate with a hydrophobic pyridoxamine derivative **3b** in the bilayer vesicle of **1a** involving copper(II) ions is a racemate (Table 1, entry 7).

(9) An acid dissociation constant for the ϵ-amino group of the lysine residue of **1a** in the bilayer state was evaluated at 30.0 °C by means of ^1H NMR titration; pK_a 9.3.

(10) A steady-state fluorescence polarization (P) value for 1,6-diphenylhexa-1,3,5-triene was 0.02 in HEPES buffer−dioxane (15:85 v/v) at 30.0 °C in the presence of **1a** and **2**, while the corresponding value for the probe embedded in the bilayer membrane was 0.35 in an aqueous HEPES buffer at the same temperature.

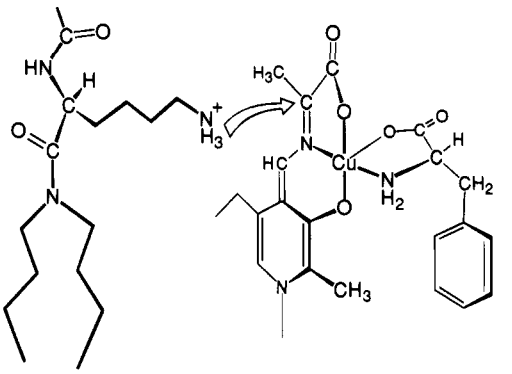

Figure 1. Protonation of the ketimine Schiff base complex, showing *si* face attack, to yield the corresponding aldimine Schiff base complex with D-Ala.

The key reaction step in forming a chiral Ala is stereoselective protonation of the quinoid−copper(II) chelate, which is the unstable intermediate present in the course of transformation from the ketimine Schiff base chelate **8** to the aldimine Schiff base chelate **9** in Scheme 1. In the light of previous studies on the structural analysis of the Schiff base chelates of vitamin B_6 derivatives, the copper(II) ion tends to assume a distorted square pyramidal configuration with phenolic oxygen, imino nitrogen, and carboxylate oxygen of the Schiff base and an additional one as basal donor atoms.[11] Although studies on the coordination geometry of the Schiff base chelate in the bilayer membrane by employing electronic absorption and ESR spectroscopy unfortunately gave no evidence for stereochemical configuration, the highest enantioselectivity observed for the transamination of L-Phe with pyruvate in the **1a**−**2**−Cu(II) system can be attributed to an analogous five-coordination geometry as shown in Figure 1, in which the L-Phe molecule present in excess over **2** acts as a chiral bidentate ligand to be bound to the quinoid−copper(II) complex, the amino nitrogen and the carboxylate oxygen being coordinated to the copper(II) ion at the fourth basal site and the axial position, respectively. In addition, the hydrophobic phenyl group of the coordinated L-Phe must be oriented in the hydrophobic inner membrane domain in a manner parallel to the lipid alkyl chains. As a result, the ϵ-ammonium group of the lysine residue of **1a** acts as a proton source to protonate the quinoid−copper(II) complex preferentially from the less hindered *si* face of the imino carbon to afford **9**.

We revealed here that the formation of a five-coordinate copper(II) complex with a chiral bidentate ligand is definitely important for producing a chiral amino acid. The transformation of a ketimine Schiff base complex to the corresponding aldimine Schiff base complex with a chiral amino acid can be assisted by an ϵ-ammonium group of the bilayer component in a manner similar to that observed for enzymic catalysis, although our artificial enzyme requires copper(II) ions unlike the natural one. The detailed characterization of supramolecular effects is now in progress in our laboratory.

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