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Enantioselective Catalysis Performed by Bilayer-type Artificial Aminotransferase. Effect of a Chiral Binaphthol Moiety Placed in the Reaction Site

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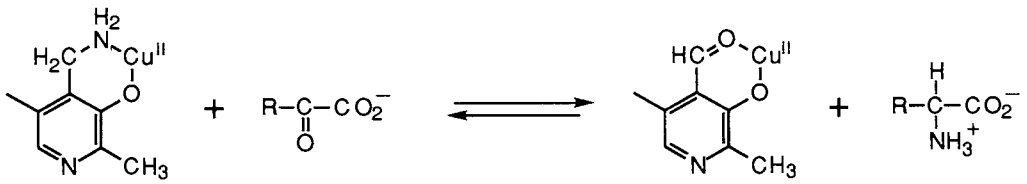
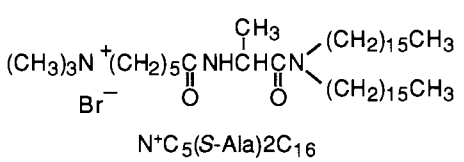
A supramolecular bilayer assembly, composed of a cationic peptide lipid having an (*S*)-alanine residue, an additional one having an (*S*)-binaphthol moiety, a hydrophobic pyridoxamine derivative, and copper(II) ions, performed substrate-selective catalysis as a vitamin B_6-dependent artificial aminotransferase to transform α-keto acids into the corresponding α-amino acids in an enantiomeric excess of the D-isomer.

Integration of synthetic molecular elements through mutual noncovalent interactions is quite useful methodology for providing various functionalized supramolecules, such as artificial receptors and enzymes.[1,2] We have recently developed supramolecular bilayer assemblies as artificial enzymes, each of which is constituted with a bilayer-forming lipid, a hydrophobic vitamin B_6 analog, and metal ions.[2] They exhibited catalytic performance as vitamin B_6-dependent artificial enzymes under mild reaction conditions, showing turnover behavior in transamination, β-replacement, and aldolase-type reactions.[3-5]

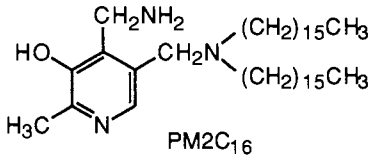
We are now focussing our work on enantioselective catalysis by these artificial enzymes. An apoprotein of a naturally occurring holoenzyme plays important roles in a specific reaction, not only providing a binding site for both coenzyme and substrate molecules but also giving a chiral microenvironment for stereospecific catalysis. In this regard, we have developed artificial enzymes having an asymmetric active site by adopting the following two different chiral components: (i) when a chiral (*S*)-histidyl residue capable of performing general acid-base catalysis is covalently introduced into a bilayer-forming lipid, the enantioselective formation of α-amino acids is observed in β-replacement and transamination reactions;[6,7] (ii) when a peptide lipid bearing an (*S*)-binaphthol moiety of axial chirality is employed, enantioselectivity is exercised in β-replacement and aldolase-type reactions.[8-10] We report here that the latter methodology is more effective than the former in transamination reactions of α-keto acids with a pyridoxamine derivative in the presence of copper(II) ions (Scheme 1).



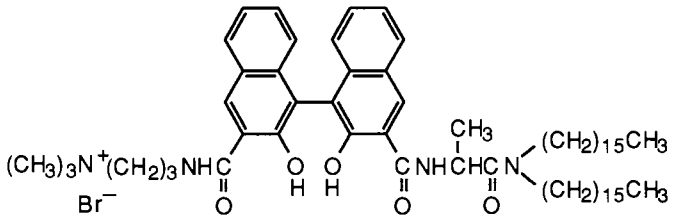
Scheme 1.



$N^+C_5(S\text{-Ala})2C_{16}$



$PM2C_{16}$



$N^+C_3(S\text{-DHBN})(S\text{-Ala})2C_{16}$

We now constituted an artificial aminotransferase by integration of a peptide lipid having an (*S*)-alanyl residue [N^+C_5(*S*-Ala)$2C_{16}$],[11] an additional one having (*S*)-binaphthol and (*S*)-alanine moieties [N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$],[8] a hydrophobic pyridoxamine analog ($PM2C_{16}$),[12] and metal ions in a molar ratio of 20 : 2 : 1 : 1 in the single-compartment vesicular state that was brought about by sonication of the mixture with a probe-type sonicator at a 30-W power for 30 s. The transamination reaction of various α-keto acids (5.0 mmol dm^{-3}) with $PM2C_{16}$ (0.050 mmol dm^{-3}) in the bilayer vesicle was carried out in an aqueous HEPES buffer (25 mmol dm^{-3}, μ 0.1 with KCl) at pH 7.0 and 30.0 °C. Enantiomers of an α-amino acid produced in each reaction mixture were treated with *o*-phthalaldehyde in the presence of D-3-mercapto-2-methylpropionic acid to give fluorescent isoindole derivatives, diastereomers to each other, and subsequently separated by HPLC on a column of Inertsil ODS-80A (GL Sciences) with methanol–acetate buffer (50 mmol dm^{-3}, pH 6.0) as eluant at volume ratios of 35 : 65 for Ala and 45 : 55 for Val, Leu, and Phe.[13]

First, we examined the transformation of 3-methyl-2-oxobutyric acid into Val as mediated by the present artificial enzyme. Marked enantioselectivity was observed, and the enantiomeric excess (e.e.) of D-Val over L-Val was found to be 40% after 1 h of incubation. Although the e.e. value gradually decreased along progress of the transamination reaction presumably due to racemization of the product, only a 2.5% decrease in the e.e. value was observed even after 7 h.

Figure 1 shows a correlation between the e.e. value and the composition of bilayer-forming lipids. In the absence of N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$, the racemic Val was obtained. The result shows that the chiral reaction site provided by the bilayer aggregate formed with N^+C_5(*S*-Ala)$2C_{16}$ alone is insufficient for the enantioselective transamination. When both N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$ and N^+C_5(*S*-Ala)$2C_{16}$ were mixed, the e.e. value evidently increased with an increase in the content of the former lipid and reached a saturation level at a 0.1 mole fraction. We have previously clarified that the transamination of an α-keto acid with $PM2C_{16}$ in the bilayer vesicle proceeds via formation of an intermediate copper(II) complex of the ketimine Schiff-base composed of these reactants, and the reaction does not occur in the absence of the metal ions.[14] On the other hand, we have revealed that the binaphthol moiety of N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$ exhibits relatively large metal-coordination ability toward copper(II) ions in the hydrophobic microenvironment provided by micelles in aqueous media.[15] These results indicate that the observed enantioselectivity is attained via formation of an intermediate ternary complex formed with the ketimine Schiff-base, N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$, and copper(II) ions.

The enantiomeric excess is also much dependent on medium pH as shown in Fig. 2. We evaluated p*K* values for the two hydroxyl groups of the binaphthol moiety of N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$ incorporated into

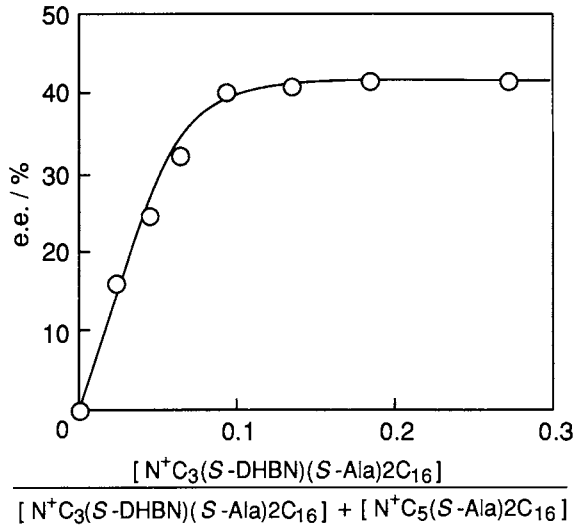


Fig. 1. Correlation between enantioselectivity for formation of Val and lipid composition as catalyzed by an artificial aminotransferase in aqueous HEPES buffer (25 mmol dm^{-3}, μ 0.1 with KCl) at pH 7.0 and 30.0 °C. Concentrations in mmol dm^{-3}: total of N^+C_5(*S*-Ala)$2C_{16}$ and N^+C_3(*S*-DHBN)(*S*-Ala)-$2C_{16}$, 1.1; $PM2C_{16}$, 0.050; $Cu(ClO_4)_2$, 0.050; 3-methyl-2-oxobutyric acid, 5.0. Enantiomeric excess (e.e.) of D-Val over L-Val.

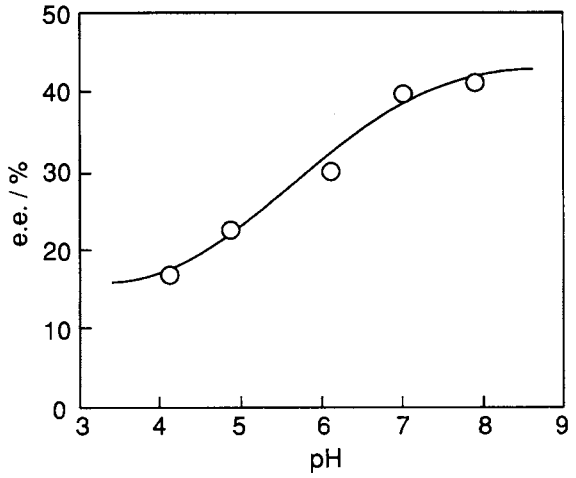


Fig. 2. pH-Dependence of enantioselectivity for formation of Val as catalyzed by an artificial aminotransferase at 30.0 °C in aqueous acetate and HEPES buffers (25 mmol dm^{-3}, μ 0.1 with KCl) at pH 4–6.5 and 7–8, respectively. Concentrations in mmol dm^{-3}: N^+C_5(*S*-Ala)$2C_{16}$, 1.0; N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$, 0.10; $PM2C_{16}$, 0.050; $Cu(ClO_4)_2$, 0.050; 3-methyl-2-oxobutyric acid, 5.0. Enantiomeric excess (e.e.) of D-Val over L-Val.

the N^+C_5(*S*-Ala)$2C_{16}$ vesicle by means of spectrophotometric titration; pK_1 = ca. 6, pK_2 = ca. 9. Thus, it seems that the coordination geometry of the intermediate ternary complex is affected by the acid dissociation equilibria to give such a pH – e.e. profile, and that higher enantioselectivity can be attained by coordination assistance of the binaphthol moiety as a potent bidentate ligand. In addition, replacement of copper(II) ions with zinc(II) or aluminium(III) ions resulted in disappearance of the enantioselectivity at pH 7.0, reflecting the importance of coordination geometry of the intermediate complex.

Table 1. Yields and Enantioselectivity Values for Transamination Catalyzed by Artificial Aminotransferase[a)]

Substrate	Product	Yield[b)]/%	e.e.[c)]/%
3-Methyl-2-oxobutyric acid	Valine	13	40
4-Methyl-2-oxovaleric acid	Leucine	12	34
Phenylpyruvic acid	Phenylalanine	24	24
Pyruvic acid	Alanine	33	15

a) In an aqueous HEPES buffer (25 mmol dm^{-3}, μ 0.1 with KCl) at pH 7.0 and 30.0 °C. Concentrations in mmol dm^{-3}: α-keto acids, 5.0; N^+C_5(*S*-Ala)$2C_{16}$, 1.0; N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$, 0.10; $PM2C_{16}$, 0.050; $Cu(ClO_4)_2$, 0.050. b) A total yield of an α-amino acid based on the amount of $PM2C_{16}$ after 1 h of incubation. c) e.e. of a D-amino acid over its L-form.

We further investigated substrate specificity by the present artificial aminotransferase. As listed in Table 1, the enantioselectivity is much dependent on stereochemical properties of β-substituents of the substrates. A molecular model study of the intermediate ternary complex suggests that presence of a bulky substituent on the β-position of the substrate induces significant steric hindrance against N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$, which deters from formation of an L-amino acid. Accordingly, the substrate bearing a bulky β-substituent is not favorable for acceleration of the transamination rate, but prevails over less hindered ones for attaining higher enantioselectivity.

In conclusion, it became apparent that the supramolecular bilayer vesicle composed of N^+C_5(*S*-Ala)$2C_{16}$, N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$, $PM2C_{16}$, and copper(II) ions exhibits catalytic performance as an artificial aminotransferase, showing marked enantioselectivity. The present artificial enzyme involving an axially chiral component in the reaction site is superior in enantiospecificity to that formed with N^+C_5(*S*-His)$2C_{16}$ having a chiral center, $PM2C_{16}$, and copper(II) ions.[7] Further research is in progress in our laboratories for development of an artificial aminotransferase which shows both enantioselective performance and turnover behavior.

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