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Enantioselective Aldolation Mediated by Functionalized Bilayer Membranes as Artificial Vitamin B_6-dependent Enzymes

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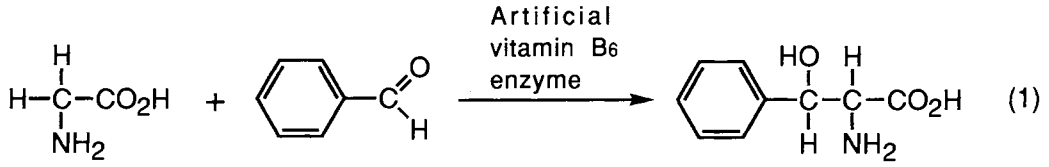
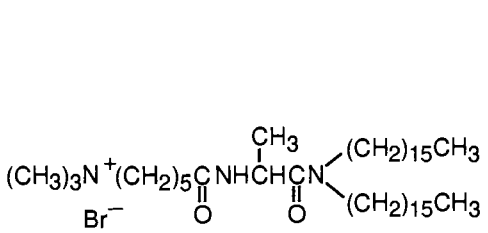
Supramolecular bilayer assemblies, each composed of a peptide lipid having an (*S*)-alanine residue, an additional one having (*S*)-binaphthol and (*S*)-alanine moieties, a hydrophobic pyridoxal derivative, and copper(II) ions, mediated the aldolation of glycine with benzaldehyde to afford β-phenylserine under mild reaction conditions, exhibiting marked enantioselectivity.

Optically active amino acids are widely utilized as chiral carbon sources for syntheses of pharmaceuticals and other functional materials, and versatile approaches to asymmetric synthesis of amino acids have been performed up to the present time.[1,2] In this regard, we have developed artificial vitamin B_6-dependent enzymes by employing bilayer assemblies of supramolecular nature, as composed of a synthetic peptide lipid, a hydrophobic vitamin B_6 derivative, and metal ions.[3] The following functional simulation of enzymatic reactions has become evident under mild reaction conditions as mediated by such supramolecular assemblies; (1) transamination between a relatively hydrophobic α-amino acid and a hydrophilic α-keto acid,[4] (2) β-replacement of serine with indoles to afford tryptophan derivatives,[5] and (3) aldolation of glycine with a hydrophobic aldehyde to yield the corresponding β-hydroxyamino acid.[6] Enantioselectivity was also observed for the transamination and β-replacement reactions by employing a chiral peptide lipid bearing a (*S*)-histidine residue or one having (*S*)-binaphthol and (*S*)-alanine moieties.[7–9] In the present work, we carried out modifications of the active site of the artificial holoenzyme previously constructed by employing various combinations of peptide lipids, hydrophobic pyridoxal derivatives, and metal ions; N^+C_5(*S*-Ala)$2C_{16}$[10] and N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$,[9] $PL2C_{16}$[11] and $PL^+C_2N2C_{16}$,[8] and copper(II) and zinc(II) ions, respectively. Then, enantioselective performance of these supramolecular assemblies was examined for the aldolation of glycine (Gly) with benzaldehyde to give β-phenylserine (β-PhSer) (refer to Eq. 1).

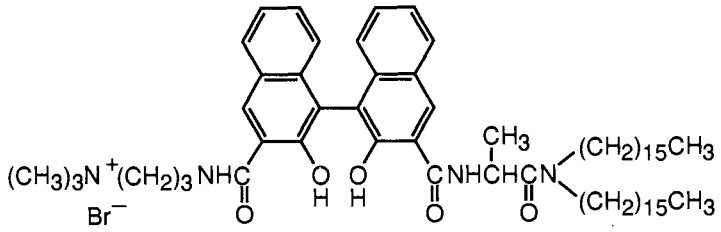
We have clarified previously[9] that the aldolation reaction shown in Eq. 1 was effectively catalyzed by a



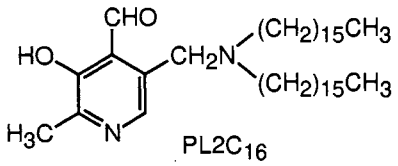
(1)



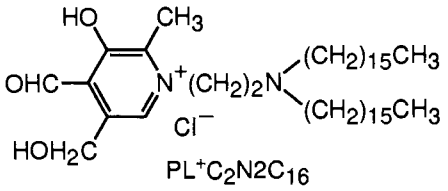
$N^+C_5(S\text{-Ala})2C_{16}$



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



$PL2C_{16}$



$PL^+C_2N2C_{16}$

supramolecular assembly composed of $N^+C_5(S\text{-Ala})2C_{16}$, $PL2C_{16}$, and zinc(II) ions in an aqueous medium at pH 7.0 and 30.0 °C. A diastereomeric ratio of *threo*-β-PhSer produced vs. the corresponding *erythro*-form was much increased in the bilayer membrane, in which the reacting species were subjected to forced molecular orientation, relative to those placed in homogeneous aqueous media. We now evaluated enantioselectivity in formation of the main product, *threo*-β-PhSer, as follows. The enantiomers of *threo*-β-PhSer, (2*R*,3*S*)- and (2*S*,3*R*)-species, in the reaction mixture were treated with *o*-phthalaldehyde in the presence of *N*-acetyl-L-cysteine to give fluorescent isoindole derivatives, which were diastereomers to each other,[12] and subsequently separated by HPLC on a column of Inertsil ODS-80A (GL Sciences) with methanol–aqueous sodium acetate (50 mmol dm^{-3}) at a 1 : 3 v/v ratio as eluant. Chromatographic fractions due to the derivatized diastereomers were identified by comparing with those observed for the identical diasteromers derived from an authentic sample of the racemic *threo*-β-PhSer. Since L-amino acid oxidase from *Crotalus durissus* is capable of converting only (2*S*,3*R*)-β-PhSer to the corresponding α-keto acid[13] and (2*R*,3*S*)-β-PhSer remains unreacted, the latter enantiomer was treated in a manner as mentioned above to obtain the corresponding isoindole derivative for its identification in the chromatographic separation.

First, we examined chiral recognition ability of a bilayer membrane formed with $N^+C_5(S\text{-Ala})2C_{16}$, $PL2C_{16}$, and zinc(II) ions in the aqueous dispersion state. However, the bilayer catalyst afforded only the racemic *threo*-β-PhSer. Even though replacement of zinc(II) ions with copper(II) ions resulted in improvement of the catalytic activity for the aldolation, only the racemic *threo*-β-PhSer was obtained under such conditions. The results reveal that the chiral reaction site provided by the bilayer aggregate of $N^+C_5(S\text{-Ala})2C_{16}$ is insufficient for the enantioselective aldolation.

Thus, we constituted a bilayer coaggregate in combination of $N^+C_5(S\text{-Ala})2C_{16}$ and $N^+C_3(S\text{-DHBN})(S\text{-Ala})2C_{16}$ to provide a chiral reaction site that is efficient for the enantioselective β-replacement reaction.[9] While the bilayer aggregate composed of $N^+C_5(S\text{-Ala})2C_{16}$, $N^+C_3(S\text{-DHBN})(S\text{-Ala})2C_{16}$, and $PL2C_{16}$ was inefficient in the enantioselective aldolation in the presence of zinc(II) ions, the identical supramolecular assembly exhibited marked enantioselectivity upon replacement of zinc(II) ions with copper(II) ions (Table 1, Entries 1 and 2). Figure 1 shows a time course for the enantiomeric excess (e.e.) of (2*S*,3*R*)-β-PhSer over (2*R*,3*S*)-β-PhSer as mediated by the supramolecular membrane in the presence of copper(II) ions. Although the e.e. value gradually decreased along progress of the aldolation, a relatively high enantioselectivity was retained at least over several

hours. Such asymmetric induction undoubtedly comes from catalytic assistance of the chiral binaphthol moiety of N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16} in the key reaction step shown in Scheme 1. A coordination geometry of the intermediate copper(II) complex, which is derived by deprotonation from the aldimine Schiff-base chelate formed with Gly, PL2C_{16}, N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16}, and copper(II) ion, is ambiguous at present. However, it is clear that the (*S*)-binaphthol moiety of the lipid involved in the Schiff-base copper(II) complex assists the *Si* attack of benzaldehyde much more favorably than the corresponding *Re* attack. In addition, a marked difference in enantioselectivity between the copper(II) and zinc(II) ion systems indicates the importance of coordination geometry around the copper(II) ion in the intermediate ternary complex.

Table 1. Yields and Enantioselectivity Values for Aldolation of Gly with Benzaldehyde as Catalyzed by Artificial Vitamin B_6 Enzymes[a)]

Entry	Supramolecular catalyst system[b)]	Yield/%[c)]	e.e./%[d)]
1	N^+C_5(*S*-Ala)2C_{16} – N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16} – PL2C_{16} – $Cu(ClO_4)_2$	18	46 (2*S*,3*R*)
2	N^+C_5(*S*-Ala)2C_{16} – N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16} – PL2C_{16} – $Zn(ClO_4)_2$	6	0
3	N^+C_5(*S*-Ala)2C_{16} – N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16} – $PL^+C_2N2C_{16}$ – $Cu(ClO_4)_2$	20	13 (2*R*,3*S*)
4	N^+C_5(*S*-Ala)2C_{16} – N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16} – $PL^+C_2N2C_{16}$ – $Zn(ClO_4)_2$	18	0

a) In HEPES buffer (10 mmol dm^{-3}, pH 7.0) at 30.0 °C, Gly (5.0 mmol dm^{-3}) and benzaldehyde (15.0 mmol dm^{-3}) were used as substrates. b) In the aqueous dispersion state. Concentrations in mmol dm^{-3}: N^+C_5(*S*-Ala)2C_{16}, 5.0; N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16}, 2.5; pyridoxal derivatives, 0.050; metal ions, 0.050. c) A total yield of *threo*-β-PhSer based on an amount of the hydrophobic pyridoxal derivative after 3 h of incubation. d) An enantiomer produced in excess is shown in parentheses.

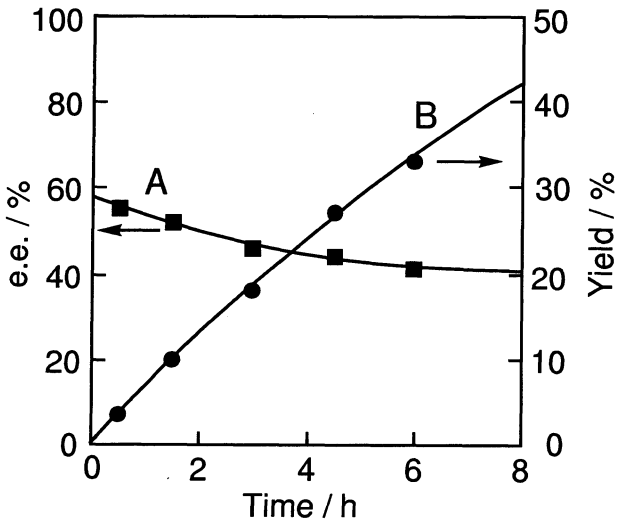
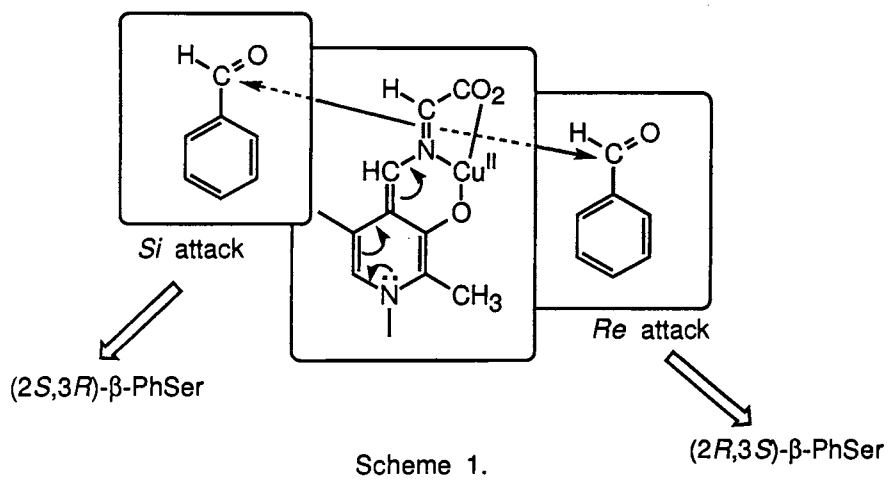


Fig. 1. Time courses for aldolation of Gly (5.0 mmol dm^{-3}) with benzaldehyde (15.0 mmol dm^{-3}) to form β-PhSer as catalyzed by an artificial enzyme constituted with N^+C_5(*S*-Ala)2C_{16} (5.0 mmol dm^{-3}), N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16} (2.5 mmol dm^{-3}), PL2C_{16} (0.050 mmol dm^{-3}), and $Cu(ClO_4)_2$ (0.050 mmol dm^{-3}) in the aqueous dispersion state in HEPES buffer (10 mmol dm^{-3}, pH 7.0) at 30.0 °C: A, enantiomeric excess (e.e.) of (2*S*,3*R*)-β-PhSer relative to (2*R*,3*S*)-β-PhSer; B, yield of *threo*-β-PhSer based on the amount of PL2C_{16}.



Scheme 1.

The enantioselective aldolation was also mediated by another bilayer assembly formed with N^+C_5(*S*-Ala)2C_{16}, N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16}, and $PL^+C_2N2C_{16}$ in the presence of copper(II) ions, but not in the presence of zinc(II) ions (Table 1, Entries 3 and 4). The opposite enantioselectivity performed by the second supramolecular assembly, as compared with that for the $PL2C_{16}$ system, is presumably due to a different stereochemical environment around the copper(II) ion that allows the *Re* attack of benzaldehyde (Scheme 1).

In conclusion, the enantioselective aldolation of Gly with benzaldehyde is mediated by supramolecular assemblies composed of two lipidic species, a hydrophobic pyridoxal, and copper(II) ions and the enantioselectivity is highly dependent on a stereochemical microenvironment around the copper(II) ion involved in the intermediate Schiff-base complex, which allows either *Si* or *Re* attack by benzaldehyde.

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