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Enantioselective β-Replacement Reaction Mediated by an Artificial Enzyme Composed of a Hydrophobic Vitamin B_6, Chiral Bilayer-forming Lipids, and Copper(II) Ions

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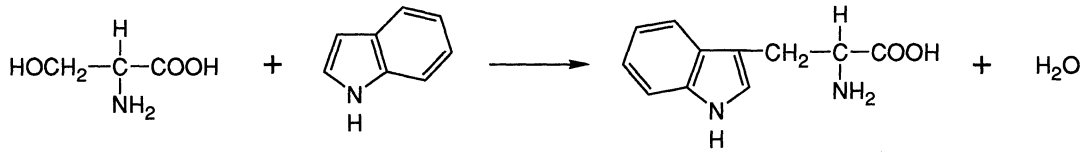
A hybrid bilayer membrane, composed of a synthetic lipid having an (*S*)-alanine residue, one having (*S*)-binaphthol and (*S*)-alanine moieties, a hydrophobic pyridoxal derivative, and copper(II) ions, exhibited a tryptophan synthase-like reactivity that affords tryptophan from serine and indole in an enantiomeric excess of the (*S*)-isomer.

Vitamin B_6 coenzyme catalyzes various transformation reactions of amino acids in specific reaction sites provided by respective apoproteins.[1] In order to simulate metabolic functions of such enzymes, we have formulated an artificial vitamin B_6-dependent enzyme with a combination of a single-walled bilayer membrane of a synthetic peptide lipid, a hydrophobic vitamin B_6, and metal ions as illustrated in Fig. 1. Tryptophan synthase, as one of vitamin B_6-dependent enzymes, catalyzes the conversion of (*S*)-serine and indole into (*S*)-tryptophan (Eq. 1). In this regard, we have previously reported that a functionalized bilayer membrane, which was composed of a cationic peptide lipid having an (*S*)-histidyl residue, a hydrophobic pyridoxal derivative, and copper(II) ions, catalyzed the β-replacement reaction of serine with indole to afford tryptophan in a significant enantiomeric excess of the (*R*)-isomer.[2,3] In the present study, we prepared novel peptide lipids, N^+C_3(*R*-DHBN)(*S*-Ala)$2C_{16}$ and N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$, in order to furnish chiral reaction sites for a hydrophobic vitamin B_6 and the substrates in the membrane system. An artificial tryptophan synthase, formed with N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$, N^+C_5(*S*-Ala)$2C_{16}$, $PL^+C_2N2C_{16}$,[3] and copper(II) ions, was found to catalyze the β-replacement reaction of DL-serine with indole to afford tryptophan in a significant (*S*)-enantiomeric excess.

N^+C_3(*R*/*S*-DHBN)(*S*-Ala)$2C_{16}$ was prepared by the reaction sequence shown in Scheme 1. 2,2'-Dihydroxy-1,1'-binaphthyl-3,3'-dicarboxylic acid (**2**) was prepared and resolved into the pure



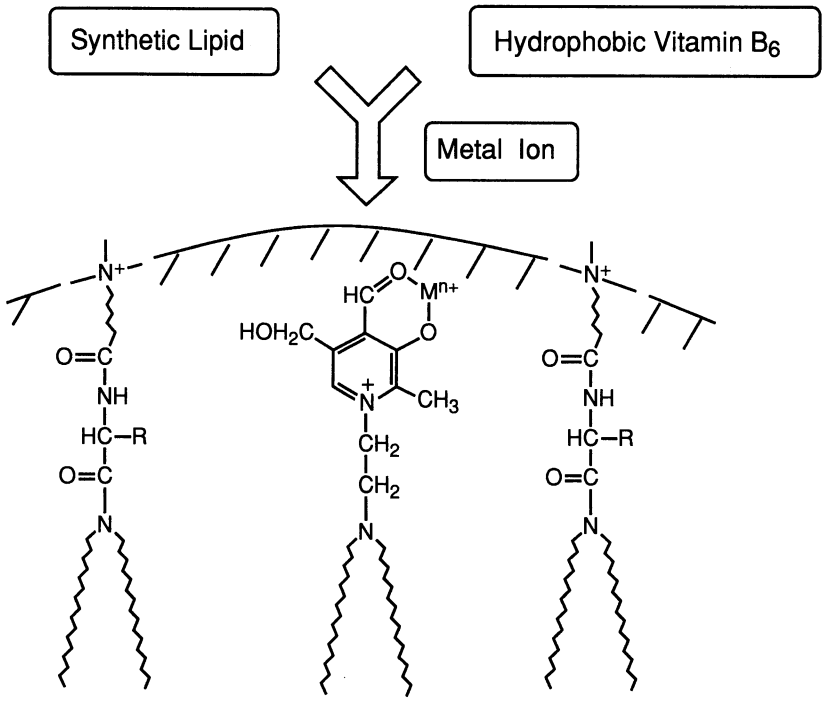
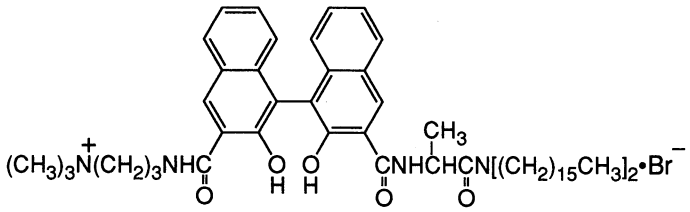


Fig. 1. Formulation of a vitamin B_6 artificial enzyme.

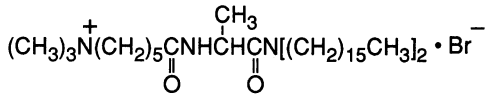


Binaphthol derivative with *R*-configuration :

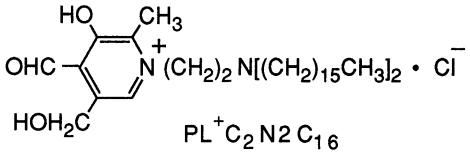
N^+C_3(*R*-DHBN)(*S*-Ala)$2C_{16}$ **(*R*)-1**

Binaphthol derivative with *S*-configuration :

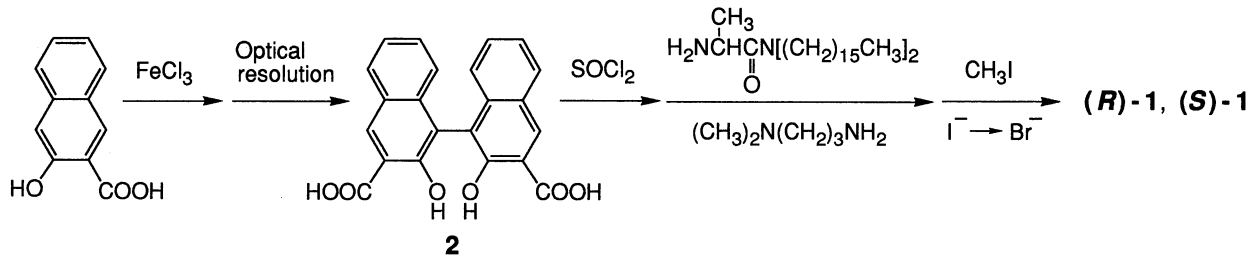
N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$ **(*S*)-1**



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



$PL^+C_2N2C_{16}$



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Scheme 1.

enantiomers according to a method reported by Cram et al.[4] Each optically pure enantiomer of **2** was converted into the corresponding acid chloride by reaction with thionyl chloride, and the resulting acid chloride underwent reaction with *N,N*-dihexadecylalaninamide[5] (a molar ratio, 1:1) at room temperature in dry benzene containing triethylamine (an equal molar amount to the acid chloride). To the reaction mixture was added an excess amount of *N,N*-dimethyl-1,3-propanediamine, and the quaternization was carried out with methyl iodide. After the iodide counterion was replaced with the bromide ion on a column of ion-exchange resin (Amberlite IRA-401) with methanol as eluant, the product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluant to give N^+C_3(*R*-DHBN)(*S*-Ala)2C_{16} and N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16} in overall yields of 22 and 32%, respectively, on the basis of an amount of **2** used. The products were identified by IR, ^{1}H-NMR, and elemental analysis. N^+C_3(*R*-DHBN)(*S*-Ala)2C_{16} Found: C, 70.78; H, 9.42; N, 5.09%. Calcd for $C_{63}H_{99}BrN_4O_5$: C, 70.56; H, 9.30; N, 5.22%. N^+C_3(*S*-DHBN)(*S*-Ala)2C_{16} Found: C, 69.74; H, 9.26; N, 5.10%. Calcd for $C_{63}H_{99}BrN_4O_5 \cdot 1/2H_2O$: C, 69.97; H, 9.32; N, 5.18%.

Single-walled vesicles in a diameter range of 300–700 Å were observed by electron microscopy applied on samples negatively stained with uranyl acetate after sonication (3 min at 30 W) of aqueous dispersion samples of N^+C_3(*R/S*-DHBN)(*S*-Ala)2C_{16}, but these sonicated samples afforded precipitates within a few hours. In addition, N^+C_3(*R/S*-DHBN)(*S*-Ala)2C_{16} alone is hardly soluble in aqueous media. Under such circumstances, we used a mixture of N^+C_3(*R/S*-DHBN)(*S*-Ala)2C_{16} and N^+C_5(*S*-Ala)2C_{16}[5] in order to obtain stable bilayer aggregates. A mixture of N^+C_3(*R/S*-DHBN)(*S*-Ala)2C_{16} and N^+C_5(*S*-Ala)2C_{16} in the dispersion state showed a sharp DSC peak caused by a phase transition from the gel to the liquid-crystalline state. The peak maximum temperature (T_m) was dependent on the mixing ratio and varied as follows: 24.9, 23.6, 22.3, 21.2, and 20.4 °C for 1:0, 9:1, 5:1, 3:1, and 2:1 molar ratios of N^+C_5(*S*-Ala)2C_{16} to N^+C_3(*R/S*-DHBN)(*S*-Ala)2C_{16}, respectively, at a total concentration of 5.0×10^{-3} mol dm^{-3}. This result indicates that N^+C_3(*R/S*-DHBN)(*S*-Ala)2C_{16} and N^+C_5(*S*-Ala)2C_{16} in their combination form stable covesicles without phase separation.

The β-replacement reaction of DL-serine with indole was carried out as follows. N^+C_3(*R/S*-DHBN)(*S*-Ala)2C_{16}, N^+C_5(*S*-Ala)2C_{16}, and $PL^+C_2N2C_{16}$, each dissolved in chloroform, were mixed, and the mixture was evaporated to dryness. An aqueous $Cu(ClO_4)_2$ [or $Zn(ClO_4)_2$] solution was added to the residue, the mixture was evaporated to dryness, and a powder sample of indole was added to the residue. DL-Serine dissolved in an acetate buffer (2.5×10^{-2} mol dm^{-3}, pH 5.0) was then added to the solid under argon atmosphere. After all the components were dispersed in the aqueous medium by Vortex mixing, the dispersion sample was sonicated with a probe-type sonicator at 30 W for 2 min under argon atmosphere to give the following final concentrations (in mol dm^{-3}) in the acetate buffer (2.5×10^{-2} mol dm^{-3}, pH 5.0) for evaluation of the reaction: $PL^+C_2N2C_{16}$, 5.0×10^{-5}; N^+C_3(*R/S*-DHBN)(*S*-Ala)2C_{16}, 1.0×10^{-4}; N^+C_5(*S*-Ala)2C_{16}, 1.0×10^{-3}; $Cu(ClO_4)_2$ [or $Zn(ClO_4)_2$], 5.0×10^{-5}; DL-serine, 5.0×10^{-3}; indole, 5.0×10^{-3}. The solution (20 mL) was deoxygenated with argon gas again, and the resulting solution was incubated at 30.0 ± 0.1 °C. Five different samples were used for evaluation of the reaction with the following combinations: (Run 1) N^+C_5(*S*-Ala)2C_{16}, $PL^+C_2N2C_{16}$, and $Cu(ClO_4)_2$; (Run 2) N^+C_3(*R*-DHBN)(*S*-Ala)2C_{16}, N^+C_5(*S*-

Ala)2C_{16}, PL$^+C_2$N2C_{16}, and $Cu(ClO_4)_2$; (Run 3) N$^+C_3$(*S*-DHBN)(*S*-Ala)2C_{16}, N$^+C_5$(*S*-Ala)2C_{16}, PL$^+C_2$N2C_{16}, and $Cu(ClO_4)_2$; (Run 4) N$^+C_3$(*R*-DHBN)(*S*-Ala)2C_{16}, N$^+C_5$(*S*-Ala)2C_{16}, PL$^+C_2$N2C_{16}, and $Zn(ClO_4)_2$; (Run 5) N$^+C_3$(*S*-DHBN)(*S*-Ala)2C_{16}, N$^+C_5$(*S*-Ala)2C_{16}, PL$^+C_2$N2C_{16}, and $Zn(ClO_4)_2$. After 200 h of the reaction time, the reaction was interrupted by adding ethylenediaminetetraacetic acid (H_4edta, 1.0 x 10^{-4} mol dm^{-3}). The reaction mixture was washed with chloroform to remove the lipids, and evaporated to dryness at room temperature. An appropriate amount of water (500 μL) was added to the residue, and the tryptophan formation was confirmed by HPLC on a column of TSK gel ODS-120T with water–methanol (7:3 v/v) as eluant. An enantiomeric excess (e.e.) of tryptophan was determined by HPLC on a column of chiral CROWNPAK CR(+) (Daicel Chemical Industries) with aqueous perchloric acid (pH 2.0) as eluant at 25 °C.

The reaction rates for all the experimental runs (Runs 1–5) were very slow, and a total yield of tryptophan for each run was a few percent, based on the amount of the hydrophobic vitamin B_6, even after 200 h of the incubation under the present experimental conditions. When the co-vesicle involving N$^+C_3$(*S*-DHBN)(*S*-Ala)2C_{16} was used (Run 3), the formation of (*S*)-tryptophan prevailed over that of the corresponding (*R*)-form by 31% e.e. However, when N$^+C_3$(*R*-DHBN)(*S*-Ala)2C_{16} was adopted as a covesicle component (Run 2), the racemic tryptophan was obtained. Even when N$^+C_3$(*S*-DHBN)(*S*-Ala)2C_{16} was used as a vesicular component, the racemic tryptophan was obtained upon addition of zinc(II) ions in place of copper(II) ions. All other kinetic runs also afforded only the racemic mixture of tryptophan. On these grounds, the enantioselectivity must be correlated with stereochemical configurations of ternary complexes composed of a Schiff-base species derived from the hydrophobic vitamin B_6 and serine,[2,3] the chiral binaphthol group in the lipid, and the copper(II) ion. The significant enantioselectivity observed in the presence of copper(II) ions is partly due to their square-planar coordination geometry in aqueous media, while zinc(II) ions are in favor of assuming a tetrahedral geometry for coordination interactions.

In conclusion, it became apparent that the covesicle formed with PL$^+C_2$N2C_{16}, N$^+C_5$(*S*-Ala)2C_{16}, N$^+C_3$(*S*-DHBN)(*S*-Ala)2C_{16}, and copper(II) ions exhibits a tryptophan synthase-like reactivity, and affords tryptophan from DL-serine and indole in an enantiomeric excess of the (*S*)-isomer under very mild conditions. Detailed mechanistic analysis for the origin of enantioselectivity is now in progress in our laboratories.

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