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β-Replacement Reaction in an Asymmetric Site Provided by an Artificial Enzyme Composed of a Hydrophobic Vitamin B_6 and Peptide Lipids

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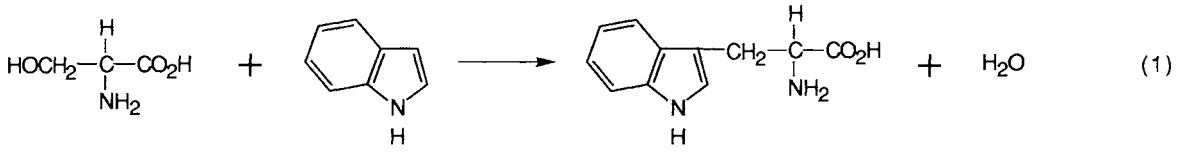
An artificial vitamin B_6 enzyme composed of a hydrophobic vitamin B_6, a peptide lipid, and an additional peptide lipid having (*S*)-binaphtol and (*S*)-alanine moieties exhibited a strong CD band in a ultraviolet region in an aqueous medium, and mediated enantioselective formation of tryptophan in the presence of bivalent copper ions as a tryptophan synthase-like reaction.

An apoprotein specific to a naturally occuring holoenzyme generally provides a binding site for both of the corresponding coenzyme and substrate, which is well separated from a bulk aqueous phase. It is quite important for construction of an artificial holoenzyme to select a molecular system that is capable of reflecting characteristic physical functions of apoproteins. Pyridoxal 5'-phosphate, a representative species of the vitamin B_6 family, catalyzes various transformations of amino acids via formation of the corresponding aldimine Schiff-bases as the initial step in specific reaction sites provided by respective apoproteins.[1] On the basis of the above concept, an artificial vitamin B_6-dependent enzyme has been constituted with a combination of a single-walled bilayer membrane composed of a synthetic peptide lipid, a hydrophobic vitamin B_6, and copper(II) ions as reported previously.[2] 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, a hydrophobic pyridoxal derivative, and copper(II) ions, catalyzed the β-replacement reaction of serine with indole to afford tryptophan as shown in Fig. 1.[3] In addition, a hybrid bilayer membrane composed of $PL^+C_2N2C_{16}$ (**1**), N^+C_5(*S*-Ala)$2C_{16}$ (**2**), N^+C_3(*S*-DHBN)(*S*-Ala)$2C_{16}$ [(*S*)-**3**], and copper(II) ions was found to exhibit a tryptophan synthase-like reactivity that affords tryptophan from DL-serine and indole in an enantiomeric excess of the (*S*)-isomer.[4] In the present study, we examined the enantioselectivity exercised by similar artificial enzymes under various conditions and clarified the origin of enantioselectivity by means of circular dichroism (CD) spectroscopy.



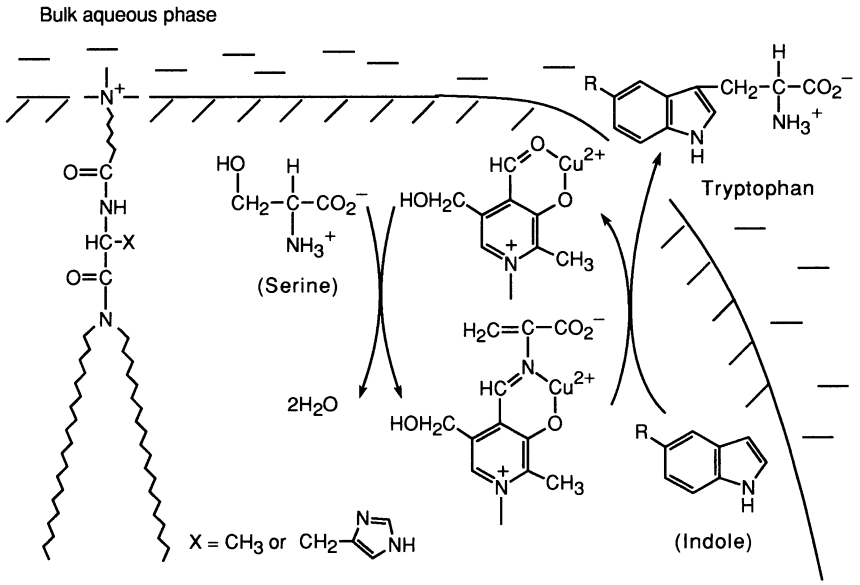
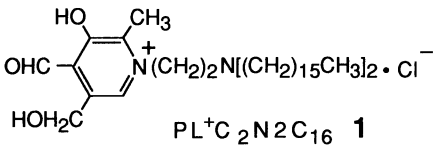
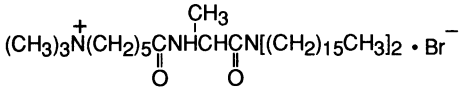


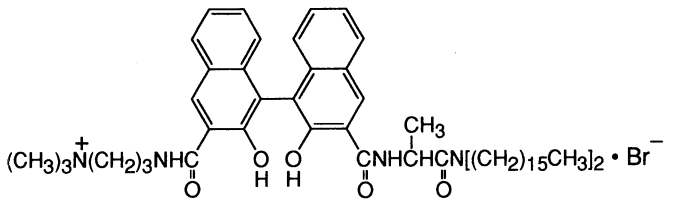
Fig. 1. Schematic representation of artificial tryptophan synthase; the artificial enzyme, as composed of peptide lipids, hydrophobic vitamin B_6, and copper(II) ions, and catalyzes the β-replacement reaction of serine with indole to afford tryptophan via formation of a Schiff-base.



$PL^{+}C_2N2C_{16}$ **1**



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



Binaphthol derivative with *R*-configuration :
$N^{+}C_3(R\text{-DHBN})(S\text{-Ala})2C_{16}$ **(*R*)-3**

Binaphthol derivative with *S*-configuration :
$N^{+}C_3(S\text{-DHBN})(S\text{-Ala})2C_{16}$ **(*S*)-3**

A typical β-replacement reaction of serine with indole was carried out as follows. Compounds **1**, **2**, and **3**, 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. 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: **1**, 5.0×10^{-5}; **2**, 1.0×10^{-3}; **3**, 1.0×10^{-4}; $Cu(ClO_4)_2$ [or $Zn(ClO_4)_2$], 5.0×10^{-5}; serine, 5.0×10^{-3}; indole, 5.0×10^{-3}. After 200 h of the reaction period, the reaction was interrupted by adding ethylenediaminetetraacetic acid (1.0×10^{-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 enantioselectivity values observed for the β-replacement reaction under various conditions are summarized in Table 1. The following findings are evident from Table 1. (i) When the covesicle involving (*S*)-**3** is used, the formation of (*S*)-tryptophan prevails over that of the corresponding (*R*)-form by *ca.* 30% e.e. In the case of low (*S*)-**3** content, the enantioselectivity is rather low (see Entry 4). The enantioselectivity for the product is independent of the chirality of serine, a substrate (see Entries 5, 6, and 7). (ii) When (*R*)-**3** is adopted as a covesicle component, the racemic tryptophan is obtained (see Entries 2 and 3). (iii) When (*S*)-**3** is used as a vesicular component, the racemic tryptophan is obtained upon addition of zinc(II) ions in place of copper(II) ions (see Entry 9).

Table 1. Enantioselectivity Exhibited by Vitamin B_6 Artificial Enzymes[a)]

		Concentrations of lipids / mol dm^{-3}					
Entry	Chirality of serine	**1**	**2**	(*R*)-**3**	(*S*)-**3**	Metal salt[b)]	Chirality of tryptophan[c)]
1	Racemate	5.0×10^{-5}	1.0×10^{-3}	———	———	$Cu(ClO_4)_2$	Racemate
2	Racemate	5.0×10^{-5}	1.0×10^{-3}	5.0×10^{-5}	———	$Cu(ClO_4)_2$	Racemate
3	Racemate	5.0×10^{-5}	1.0×10^{-3}	1.0×10^{-4}	———	$Cu(ClO_4)_2$	Racemate
4	Racemate	5.0×10^{-5}	1.0×10^{-3}	———	5.0×10^{-5}	$Cu(ClO_4)_2$	*S*-Isomer (20% e.e.)
5	Racemate	5.0×10^{-5}	1.0×10^{-3}	———	1.0×10^{-4}	$Cu(ClO_4)_2$	*S*-Isomer (31% e.e.)
6	*R*-Isomer	5.0×10^{-5}	1.0×10^{-3}	———	1.0×10^{-4}	$Cu(ClO_4)_2$	*S*-Isomer (30% e.e.)
7	*S*-Isomer	5.0×10^{-5}	1.0×10^{-3}	———	1.0×10^{-4}	$Cu(ClO_4)_2$	*S*-Isomer (30% e.e.)
8	Racemate	5.0×10^{-5}	1.0×10^{-3}	1.0×10^{-4}	———	$Zn(ClO_4)_2$	Racemate
9	Racemate	5.0×10^{-5}	1.0×10^{-3}	———	1.0×10^{-4}	$Zn(ClO_4)_2$	Racemate

a) In aqueous acetate buffer (2.5×10^{-2} mol dm^{-3}, pH 5.0), serine (5.0×10^{-3} mol dm^{-3}) and indole (5.0×10^{-3} mol dm^{-3}) were used as substrates. b) Concentration, 5.0×10^{-5} mol dm^{-3}. c) The reaction rates for all the experiments were very slow, and a total yield of tryptophan for each run was a few percent, based on the amount of **1**, after 200 h of incubation under the present experimental conditions. An enantiomeric excess of tryptophan was determined by HPLC.

In order to characterize the stereochemical microenvironments provided by the (*R*)-**3**-containing covesicle and the (*S*)-**3**-containing one, CD spectroscopy was applied to these molecular assemblies in an acetate buffer (2.5×10^{-2} mol dm^{-3}, pH 5.0) containing hexylamine, as shown in Fig. 2. Both solutions are not CD active in a wavelength range greater than 400 nm, and strong CD bands arising from the chiral binaphthyl moiety are observed in the 200–400 nm range. A quite strong CD band is observed at *ca.* 250 nm for the covesicle containing (*S*)-**3**. On the other hand, the corresponding CD pattern for the covesicle containing (*R*)-**3** is reverse to that for the other covesicle in ellipticity sign and shows rather weak intensity. Since the absolute value of CD band intensity for (*S*)-**3** in acetonitrile is comparable to those for (*R*)-**3** in acetonitrile and in aqueous media, the significant increase in the Cotton effect observed for (*S*)-**3** in an aqueous medium must originate from the formation of a specific molecular assembly. On the basis of theoretical analysis of CD spectra for 1,1'-

binaphthalene derivatives, the Cotton effect is expected to appear in a 250 nm range, as caused by ^1B_b transitions of the naphthalene chromophores.[5] In addition, an intensity of the Cotton effect originated from the ^1B_b transition evidently decreases as the dihedral angle between the naphthalene planes of the 1,1'-binaphthalene skeleton.[5] Since the CD ellipticity values for both solutions are different from each other and the value for the (*S*)-**3**-containing covesicle is much stronger than the other, the former covesicle is capable of providing an efficient stereochemical microenvironment for enantioselectivity.

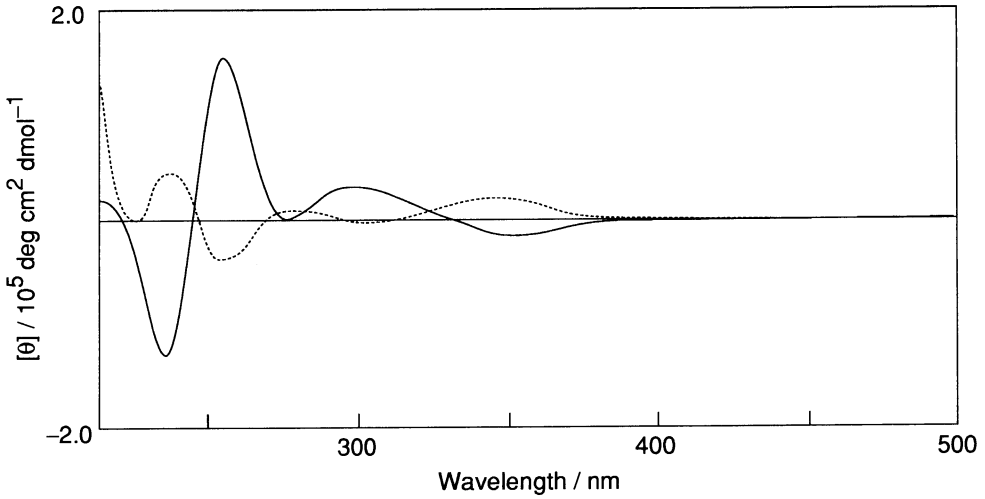


Fig. 2. CD spectra for artificial tryptophan synthase composed of synthetic lipids, a hydrophobic pyridoxal derivative, copper(II) ions, and hexylamine (5.0×10^{-3} mol dm^{-3}) in acetate buffer (2.5×10^{-2} mol dm^{-3}, pH 5.0) at 30 °C. Solid line: **1**, 5.0×10^{-5}; **2**, 1.0×10^{-3}; (*S*)-**3**, 1.0×10^{-4}, Cu^{2+}, 5.0×10^{-5} mol dm^{-3}. Dotted line: **1**, 5.0×10^{-5}; **2**, 1.0×10^{-3}; (*R*)-**3**, 1.0×10^{-4}, Cu^{2+}, 5.0×10^{-5} mol dm^{-3}.

In conclusion, it became apparent that the covesicle formed with **1**, **2**, (*S*)-**3**, and copper(II) ions exhibits a tryptophan synthase-like reactivity and affords tryptophan from serine and indole in an enantiomeric excess of the (*S*)-isomer under very mild conditions, regardless of the chirality of serine. The difference in enantioselectivity between the covesicles containing (*R*)-**3** and (*S*)-**3** seems to originate from the difference in conformational status of the binaphthalene moiety embedded in the respective covesicles.

References

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