Functionalized Bilayer Membranes as Artificial Tryptophan Synthase. Characterization of Catalytic Efficiency, Substrate Specificity, and Reaction Selectivity†

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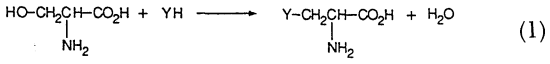
(Received April 10, 1990)

Functionalized bilayer membranes having vitamin B_6 activity effectively catalyzed β-replacement reactions of serine with indoles to afford the corresponding tryptophan derivatives in aqueous media under mild conditions. Catalytic capability of the present artificial enzyme was subjected to change by changing a combination of molecular components constituting the catalyst system. The structural mode of a hydrophobic pyridoxal derivative as the coenzyme model, the catalytic ability of an amino acid residue placed in a peptide lipid which forms single-walled bilayer vesicles as the apoenzyme model, and the coordination property of added metal ions were found to be responsible for the overall catalytic performance. Multifunctional assistance was observed in the β-replacement reaction of serine with indole, and the reaction proceeded in preference to other side reactions, such as β-elimination, dealdolation, and transamination reactions. Substrate selectivity was found to be primarily dependent on the nucleophilicity of indole derivatives.

In biological systems, vitamin B_6-dependent enzymes are primarily involved in metabolisms of α-amino acids. These enzymatic reactions are classified into several reaction types; such as transamination, racemization, decarboxylation, β- and γ-elimination, β- and γ-replacement, and dealdolation reactions. Among these reactions, β-replacement of an α-amino acid with a nucleophile is very attractive from the viewpoint of synthetic organic chemistry, because various β-substituted alanines may be prepared from a simple α-amino acid, such as serine, and nucleophiles (YH) (refer to Eq. 1). Recent growing interest in biomi-



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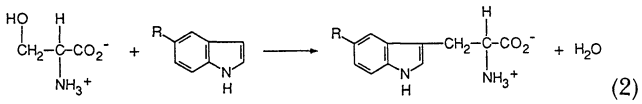
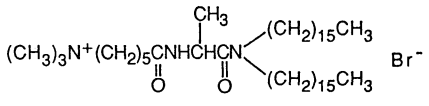
metic chemistry has led us to develop several artificial enzymes which were constituted with simpler molecular devices in comparison with the corresponding natural enzymes and capable of simulating catalytic functions of vitamin B_6-dependent holoenzymes.[1-7] Few artificial catalysts effective in β-replacement reactions of α-amino acids have been reported up to the present time. In 1950's, Snell et al. found that the β-replacement reaction of serine with indole, which resulted in formation of tryptophan, proceeded nonenzymatically to a slight extent in aqueous media in the presence of pyridoxal and aluminum(III) ions under severe temperature conditions (100 °C).[8] In recent years, Breslow et al. developed coenzyme-bound cyclodextrins as vitamin B_6-dependent artificial enzymes. These catalysts were effective as a transaminase mimic,[3] but not so much as a tryptophan synthase mimic.[5]

We have recently developed functionalized bilayer membranes, each being constituted with a synthetic peptide lipid and a hydrophobic vitamin B_6 derivative, as vitamin B_6-dependent holoemzyme models.[9-13] In the presence of copper(II) ions, these holoenzyme models catalyzed the transamination reaction of an α-amino acid with an α-keto acid under mild conditions, exhibiting high substrate selectivity.[14-16] We now examined the reactivity of similar membrane catalysts in the β-replacement reaction of serine with indoles (refer to Eq. 2). We performed modifications

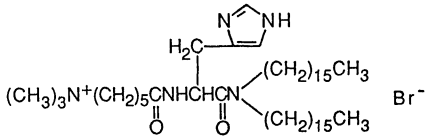


(2)

of the active site of our artificial holoenzyme by employing various combinations of peptide lipids, $N^+C_5Ala2C_{16}$ and $N^+C_5His2C_{16}$, and hydrophobic pyridoxal derivatives, $PL2C_{16}$ and PL^+2C_{16}. In this article, we are to report on catalytic activity, substrate selectivity, and reaction selectivity exercised by such functionalized membranes as tryptophan synthase mimics.[17]



$N^+C_5Ala2C_{16}$



$N^+C_5His2C_{16}$

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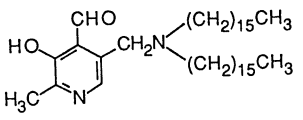
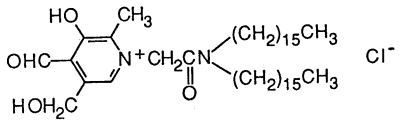


PL2C16



PL+2C16

Experimental

Materials. The following α-amino acids and their derivatives were obtained from commercial sources as guaranteed reagents and used without further purification: L-serine, L-tryptophan, 5-hydroxy-L-tryptophan, 5-methyl-DL-tryptophan, 5-methoxy-DL-tryptophan (these from Nacalai Tesque), benzyl L-serinate, and benzyl L-tryptophanate (these from Sigma Chemical Co., Missouri, U.S.A). Indole and its 5-substituted derivatives, as nucleophiles for the β-replacement reaction of α-amino acids, were commercially available as guaranteed reagents: indole, 5-hydroxyindole, 5-methylindole, and 5-methoxyindole (all from Nacalai Tesque). Preparation and characterization of 1-[(dihexadecylcarbamoyl)methyl]-4-formyl-3-hydroxy-5-hydroxymethyl-2-methylpyridinium chloride (PL^+2C_{16}),[13] *N,N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{16}$),[18] and *N,N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5His2C_{16}$)[12] have been reported elsewhere.

5-[(Dihexadecylamino)methyl]-4-formyl-2-methyl-3-pyridinol ($PL2C_{16}$) was prepared from 4-aminomethyl-5-[(dihexadecylamino)methyl]-2-methyl-3-pyridinol[10] by transamination with α-oxoglutaric acid in methanol in the presence of zinc nitrate, in a manner similar to that reported for the synthesis of 5-dodecylthiomethyl-4-formyl-2-methyl-3-pyridinol:[9] a yellow solid (yield 58%). 400MHz ^{1}H NMR ($CDCl_3$) δ=0.87 [6H, t, $CH_2CH_2(CH_2)_{13}C\underline{H}_3$], 1.25 [52H, m, $CH_2CH_2(C\underline{H}_2)_{13}CH_3$], 1.89 [4H, m, $CH_2C\underline{H}_2(CH_2)_{13}CH_3$], 2.53 [3H, s, CH_3 on pyridine ring], 3.49 [4H, m, $C\underline{H}_2CH_2(CH_2)_{13}CH_3$], 3.67 [2H, s, CH_2N on pyridine ring], 7.96 [1H, s, H on pyridine ring], 8.07 [1H, s, CHO].

Found: C, 72.63; H, 11.65; N, 4.24%. Calcd for $C_{40}H_{74}N_2O_2 \cdot HCl \cdot 0.5H_2O$: C, 72.74; H, 11.60; N, 4.24%.

Copper(II) perchlorate (Kishida Chemical Co.), aluminum(III) nitrate (Wako Pure Chemical Industries), gallium(III) nitrate (Ishizu Seiyaku Co.), and zinc(II) perchlorate (Kishida Chemical Co.) were dissolved in deionized water and standardized by conventional chelatometric titration. Disodium ethylenediaminetetraacetate (EDTA; Dojin Chemical Laboratories) was obtained as a guaranteed reagent.

General Analyses and Measurements. Elemental analyses were performed at the Microanalysis Center of Kyushu University. 400 MHz ^{1}H NMR spectra were taken on a JEOL JNM-GSX400 spectrometer installed at the Center of Advanced Instrumental Analysis of Kyushu University. A Beckman Φ71 pH meter equipped with a Beckman 39505 combined electrode was used for pH measurements.

Kinetic Measurements. Single-walled covesicles composed of the peptide lipid and the hydrophobic pyridoxal derivative were prepared upon sonication of an aqueous dispersion sample of these species by a probe-type sonicator (W-220F, Heat Systems-Ultrasonics) in a manner similar to that reported previously.[14] Progress of the β-replacement reaction of L-serine or benzyl L-serinate with an indole derivative, as catalyzed by the vesicular catalyst in aqueous buffers at 30.0 °C in the presence and absence of copper(II) ions, was monitored by measuring amounts of the corresponding tryptophan derivative produced in the course of reaction. Tryptophans were separated by liquid chromatography on a column of TSK gel ODS-120T (length, 250 mm; internal diameter, 4.6 mm) by employing a HPLC system assembled with a Hitachi 655A-11 pump, a Hitachi L-6000 pump, and a Hitachi L-5000 LC controller, and eluting fractions were monitored by a Hitachi F-1000 fluorescence spectrophotometer: flow rate, 1.0 mL min^{-1}; mobile phase, mixtures of Tris buffer (10 mmol dm^{-3}, pH 8.0) and methanol at volume ratios of 7:3, 4:1, and 1:1 for tryptophan and 5-methoxytryptophan, 5-hydroxytryptophan, and 5-methyltryptophan, respectively. A column of TSK gel SILICA-150 (length, 250 mm; internal diameter, 4.6 mm) was used for separation of benzyl tryptophanate: flow rate, 1.0 mL min^{-1}; mobile phase, methanol. The β-elimination product derived from serine, pyruvate, was converted into the fluorescent 3-methyl-2-quinoxalinol by reaction with *o*-phenylenediamine in a manner similar to that reported in literature,[19] and analyzed by the HPLC system: column, TSK gel ODS-120T; flow rate, 1.0 mL min^{-1}; mobile phase, a mixture of aqueous tetrapropylammonium bromide (0.1 mol dm^{-3}), aqueous sodium phosphate (0.1 mol dm^{-3}, pH 7.0), water, and acetonitrile at a volume ratio of 2:10:7:1. The dealdolation product of serine, glycine, was dansylated[14] and analyzed by the HPLC system: column, TSK gel ODS-120T; flow rate, 1.0 mL min^{-1}, mobile phase, a mixture of Tris buffer (10 mmol dm^{-3}, pH 8.0) and methanol at a volume ratio of 7:3. Progress of the transamination reaction of serine with pyruvate was monitored by measuring amounts of alanine formed in the course of reaction without indole. The transamination product, alanine, was dansylated[14] and analyzed by the HPLC system: column, TSK gel ODS-120T; flow rate, 1.0 mL min^{-1}; mobile phase, a mixture of Tris buffer (10 mmol dm^{-3}, pH 8.0) and methanol at a volume ratio of 2:1. The reactivity was evaluated from the initial reaction rates determined by measuring amounts of the corresponding products formed in a period of the first 16 h.

Results

Catalytic Efficiency. In our preliminary communication,[17] we have reported that synthetic bilayer vesicles having vitamin B_6 activity markedly enhanced the β-replacement reaction of L-serine (L-Ser) with indole to afford tryptophan (Trp), showing turnover behavior of the catalyst, in aqueous media under mild conditions. As listed in Table 1, the rate enhancement in the overall reaction mainly comes from three functional factors; (i) a medium effect provided by the bilayer membrane in aqueous media, (ii) a general acid-base catalysis by the imidazolyl group of the

Table 1. Catalytic Activities of Various Catalysts for β-Replacement Reaction of L-Ser with Indole at 30.0 °C

Catalyst[a]	Relative reactivity[b]
PL^+2C_{16}-$N^+C_5His2C_{16}$-Cu^{II}	180
PL^+2C_{16}-$N^+C_5His2C_{16}$	130
PL^+2C_{16}-$N^+C_5Ala2C_{16}$-Cu^{II}	60
PL^+2C_{16}-$N^+C_5Ala2C_{16}$	51
Pyridoxal-Cu^{II}	1.2
Pyridoxal	1[c]

a) In an aqueous acetate buffer (25 mmol dm^{-3}, μ 0.04 with KCl, pH 5.0). Concentrations in mmol dm^{-3}: L-Ser, 5.0; indole, 5.0; pyridoxal derivatives, 0.025; peptide lipids, 1.0; $Cu(ClO_4)_2$, 0.050. For metal-free system, EDTA (0.10 mmol dm^{-3}) was added. b) Analyzed by HPLC for Trp and evaluated on the basis of initial reaction rates. c) Initial rate, 1.8×10^{-6} mmol dm^{-3} h^{-1}.

Table 2. Metal-ion Effect in β-Replacement Reaction of L-Ser with Indole in the PL^+2C_{16}-$N^+C_5His2C_{16}$ Vesicle at 30.0 °C[a]

Metal ion	Relative reactivity[b]
Cu^{II}	2.6
Al^{III}	1.8
Ga^{III}	1.8
Zn^{II}	1.4
None[c]	1[d]

a) In an aqueous acetate buffer (25 mmol dm^{-3}, μ 0.04 with KCl, pH 5.0). Concentrations in mmol dm^{-3}: L-Ser, 50; indole, 50; PL^+2C_{16}, 0.50; $N^+C_5His2C_{16}$, 10; metal ions, 0.50. b) Analyzed by HPLC for Trp and evaluated on the basis of initial reaction rates. c) EDTA (0.10 mmol dm^{-3}) was added. d) Initial rate, 0.12 mmol dm^{-3} h^{-1}.

peptide lipid ($N^+C_5His2C_{16}$), and (iii) a coordination effect by metal ions. Thus, the vesicular catalyst composed of PL^+2C_{16}, $N^+C_5His2C_{16}$, and copper(II) ions exhibited the highest activity for the β-replacement reaction, showing turnover behavior.[17] Addition of other metal ions, such as aluminum(III), gallium(III), and zinc(II) ions, resulted in rate enhancement to certain extents in comparison with the reaction without metal ions (Table 2).

The catalytic activity of the PL^+2C_{16}-$N^+C_5His2C_{16}$ vesicular system in the β-replacement of L-Ser with indole at pH 5.0 was higher than that at pH 9.5 in the presence and absence of metal ions (Table 3). The catalytic activity of $PL2C_{16}$ in the metal-free $N^+C_5His2C_{16}$ vesicle was comparable to that of PL^+2C_{16} in the identical vesicle at pH 5.0, but much lower at pH 9.5 (Table 3).

Substrate Specificity. As for the β-replacement reaction carried out in the metal-free PL^+2C_{16}-$N^+C_5His2C_{16}$ vesicular system, L-Ser and its hydrophobic benzyl ester derivative were comparable to each other in reactivity (Table 4). On the other hand, marked substrate specificity was observed for indole derivatives as nucleophiles in the presence of copper-

Table 3. Catalytic Activities of $N^+C_5His2C_{16}$ Vesicles Having Vitamin B_6 Activity for β-Replacement Reaction of L-Ser with Indole at 30.0 °C[a]

Pyridoxal derivative	Metal ion	pH[b]	Relative reactivity[c]
PL^+2C_{16}	Cu^{II}	5.0	31
		9.5	18
PL^+2C_{16}	None	5.0	9.6
		9.5	4.7
$PL2C_{16}$	None	5.0	7.6
		9.5	1[d]

a) Concentrations in mmol dm^{-3}: L-Ser, 5.0; indole, 5.0; pyridoxal derivatives, 0.05; $N^+C_5His2C_{16}$, 1.0; $Cu(ClO_4)_2$, 0.05. For metal-free system, EDTA (0.10 mmol dm^{-3}) was added. b) In an aqueous acetate buffer (25 mmol dm^{-3}, μ 0.10 with KCl) at pH 5.0, and in an aqueous carbonate buffer (20 mmol dm^{-3}, μ 0.10 with KCl) at pH 9.5. c) Analyzed by HPLC for Trp and evaluated on the basis of initial reaction rates. d) Initial rate, 9.0×10^{-6} mmol dm^{-3} h^{-1}.

Table 4. Substrate Specificity of Artificial Tryptophan Synthase Formed with PL^+2C_{16} and $N^+C_5His2C_{16}$ at 30.0 °C[a]

Serine derivative	pH[b]	Relative reactivity[c]
Benzyl L-serinate	5.0	2.4
	10.0	1.4
L-Ser	5.0	2.0
	10.0	1[d]

a) Concentrations in mmol dm^{-3}: L-Serine derivatives, 5.0; indole, 5.0; pyridoxal derivatives, 0.05; $N^+C_5His2C_{16}$, 1.0; EDTA, 0.10. b) In an aqueous acetate buffer (25 mmol dm^{-3}, μ 0.10 with KCl) at pH 5.0, and in an aqueous carbonate buffer (20 mmol dm^{-3}, μ 0.10 with KCl) at pH 10.0. c) Analyzed by HPLC for Trp and evaluated on the basis of initial reaction rates. d) Initial rate, 4.3×10^{-5} mmol dm^{-3} h^{-1}.

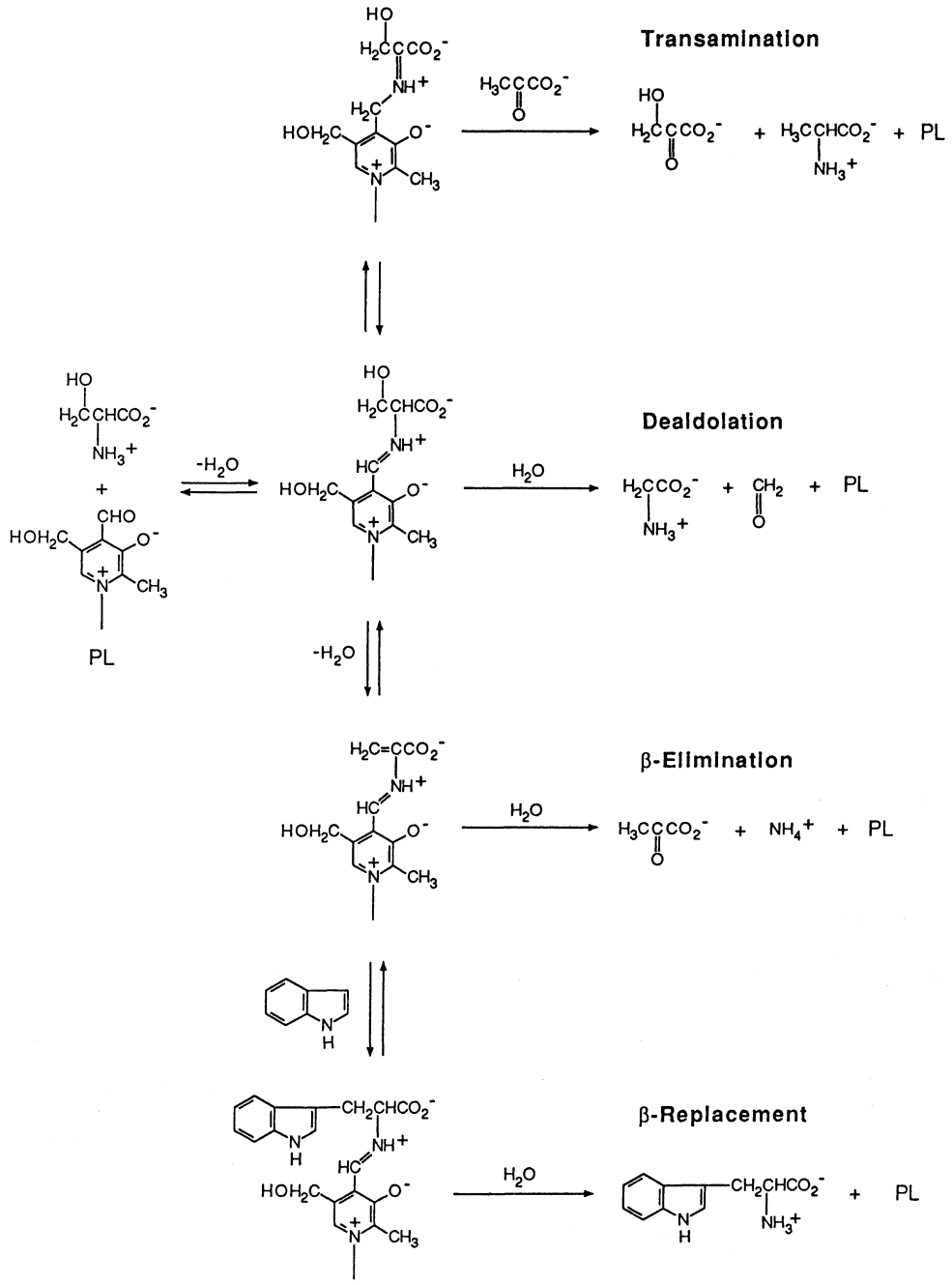
Table 5. Substrate Specificity of Artificial Tryptophan Synthase Formed with PL^+2C_{16}, $N^+C_5His2C_{16}$, and Copper(II) Ions, and Natural Tryptophanase at 30.0 °C

Indole derivative	Relative reactivity	
	Artificial enzyme[a]	Natural enzyme[b]
5-Hydroxyindole	40	0.04
5-Methylindole	18	0.04
5-Methoxyindole	13	0
Indole	1[c]	1[d]

a) In aqueous media at pH 4.2. Concentrations in mmol dm^{-3}: L-Ser, 5.0; indole derivatives, 5.0; pyridoxal derivatives, 0.0071; $N^+C_5His2C_{16}$, 1.0; $Cu(ClO_4)_2$, 0.05. Analyzed by HPLC for tryptophan derivatives and evaluated on the basis of initial reaction rates. b) Taken from Ref. 20. In an aqueous ammonium chloride buffer (100 mmol dm^{-3}) at pH 9.0. Amounts in a total volume of 2 ml: potassium pyruvate, 150 μmol; indole derivatives, 100 μmol; pyridoxal-5′-phosphate, 0.2 μmol; enzyme, 0.179 unit. c) Initial rate, 3.38×10^{-5} mmol dm^{-3} h^{-1} d) Yield, 1.60 μmol/20 min.

(II) ions (Table 5). The reactivity of indoles in the β-replacement of L-Ser increased in the following order: indole<5-methoxyindole<5-methylindole<5-hydroxyindole. Substrate specificity for the identical series of indole substrates as demonstrated by a natural enzyme, tryptophanase,[20] is also shown in Table 5.

Reaction Selectivity. Since the $PL^{+}2C_{16}$-$N^{+}C_{5}$-$His2C_{16}$ bilayer vesicle exhibited the highest catalytic activity as an artificial tryptophan synthase in the presence of copper(II) ions, its selectivity for other side reactions (refer to Scheme 1) was examined as summarized in Table 6. As clarified previously, the present vesicular catalyst behaves as an effective artificial transaminase for the transamination reaction of a hydrophobic α-amino acid with a hydrophilic α-keto acid, showing high substrate selectivity.[14] In other words, the catalytic activity in the amino group transfer from a hydrophilic α-amino acid to an α-keto acid is extremely low. Indeed, the transamination reaction of L-Ser with pyruvate was much repressed (Table 6). The dealdolation reaction of L-Ser to afford glycine and formaldehyde was a minor pathway with the present vesicular catalyst. Although the β-elimination reaction of L-Ser to give pyruvate and ammonia proceeded to a certain extent, a major catalytic reaction was referred to the β-replacement of L-



Scheme 1.

Table 6. Reaction Selectivity of Artificial Vesicular Catalyst Formed with $PL^{+}2C_{16}$, $N^{+}C_5His2C_{16}$, and Copper(II) Ions at 30.0 °C [a]

pH[b]	Relative reactivity[c]			
	β-Replacement	β-Elimination	Dealdolation	Transamination
5.0	150	70	1[d]	7.3
7.0	110	53		
9.5	77	40		

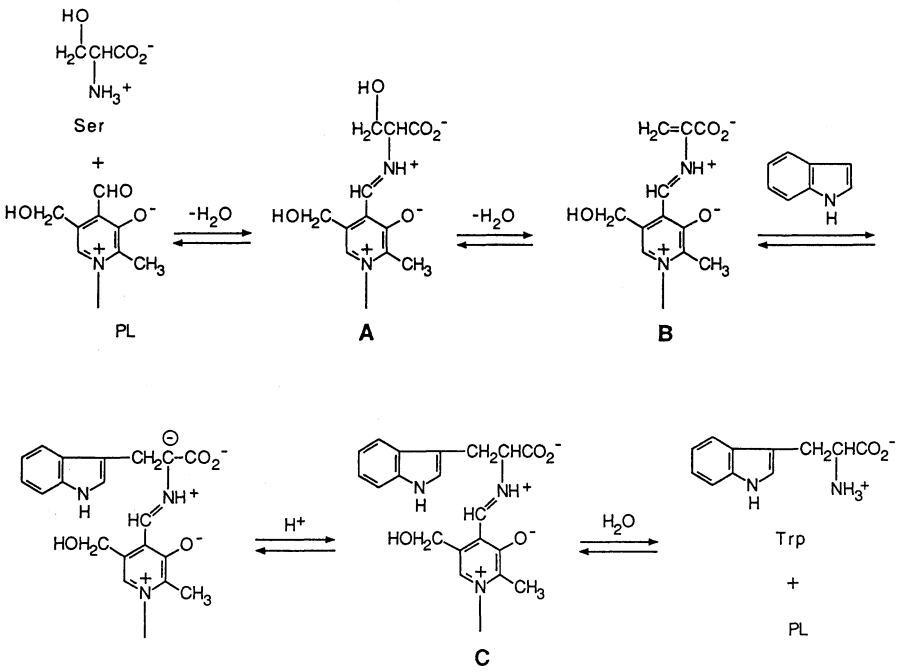
a) Concentrations in mmol dm^{-3}: L-Ser, 5.0; indole, 5.0; $PL^{+}2C_{16}$, 0.05; $N^{+}C_5His2C_{16}$, 1.0; $Cu(ClO_4)_2$, 0.05. b) In an aqueous acetate buffer (25 mmol dm^{-3}, μ 0.01 with KCl) at pH 5.0, in an aqueous HEPES buffer (25 mmol dm^{-3}, μ 0.10 with KCl) at pH 7.0, and in an aqueous carbonate buffer (20 mmol dm^{-3}, μ 0.10 with KCl) at pH 9.5. c) Analyzed by HPLC for Trp, pyruvate, and glycine obtained by β-replacement, β-elimination, and dealdolation reactions, respectively. The transamination reaction of serine with pyruvate (5.0 mmol dm^{-3}) was monitored without indole; analyzed by HPLC for alanine. Reactivity parameters were evaluated on the basis of initial reaction rates. d) Initial rate, 3.0×10^{-6} mmol dm^{-3} h^{-1}.

Ser with indole.

Discussion

In the present vesicular system, the β-replacement reaction of Ser with indole proceeded to afford Trp plausibly via reaction steps shown in Scheme 2, in a manner analogous to that proposed for tryptophan synthase and tryptophanase.[21-23] The aldimine Schiff-base (**A** in Scheme 2), derived from the pyridoxal derivative (PL) and Ser, is a common intermediate in the vitamin B_6-catalyzed reactions. In order to achieve formation of Trp, the effective nucleophilic attack of indole on the α,β-elimination product (**B** in Scheme 2), formed by dehydration of **A**, must be followed by the hydrolysis of the aldimine Schiff-base (**C** in Scheme 2). The first and second steps in Scheme 2 are dehydration processes and seem to be thermodynamically unfavorable in homogeneous aqueous media. When Schiff-base **B** was hydrolyzed prior to the attack by indole, the reaction results in β-elimination to give pyruvate, ammonia, and the PL derivative.

Snell et al. reported that the β-elimination of Ser, as catalyzed by pyridoxal and aluminum(III) ions, readily took place in aqueous media in the absence of indole under severe reaction conditions (100 °C and pH 6).[24] Upon addition of indole to the reaction system, only a small amount of Trp (ca. 1 % yield) was obtained as the β-replacement product under the identical conditions.[8] Thus, an efficient attack of indole on the intermediate species (**B**) seems to be a key step for the β-replacement reaction. Recently, Breslow et al. found that formation of Trp from β-chloroalanine and indole was catalyzed by an artificial catalyst, a pyridoxamine moiety bound to β-cyclodextrin, in the

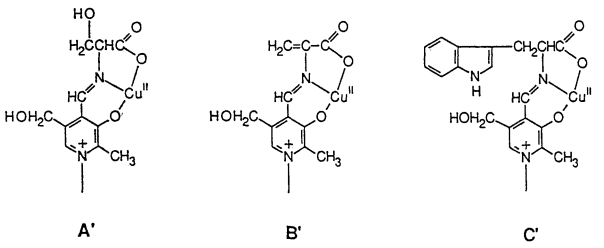


Scheme 2.

presence of aluminum(III) ions under severe conditions similar to those adopted by Snell et al.[5] An efficient attack of indole on the intermediate (equivalent to **B**) may be favored, since the β-cyclodextrin cavity provides a hydrophobic binding site for the indole molecule. However, the catalytic activity of their macrocyclic enzyme model as an artificial tryptophan synthase was not so significant: a yield of Trp was somewhat improved but only a few percent.

In the present work, we adopted mild reaction conditions (30.0 °C and pH 5—10) for investigation of the β-replacement of Ser derivatives with indoles by the vitamin B_6-dependent vesicular catalysts. Even under such mild conditions, the catalyst composed of PL^+2C_{16}, $N^+C_5His2C_{16}$, and copper(II) ions exhibited significantly high activity for the formation of Trp from Ser and indole, showing turnover behavior.[17] In the light of kinetic data listed in Table 1, this catalyst system must exercise three kinds of catalytic functions cooperatively to achieve the best performance; (i) a microenvironmental effect of hydrophobic nature provided by the bilayer vesicle, (ii) a general acid-base catalysis by the imidazolyl group of the peptide lipid, and (iii) a coordination effect by copper(II) ions. These catalytic functions have also been evidenced in the transamination reaction of α-amino acids with α-keto acids as catalyzed by the same vesicular catalyst.[10-16] A microenvironmental polarity at the reaction site situated in the intramembrane domain was evaluated to be equivalent to 2-propanol [$E_T(30)$=50.7 kcal mol^{-1}].[11] The relatively hydrophobic reaction site acts to eliminate water molecules significantly, so that the formation of intermediates **A** and **B** is much favored. In addition, indole is much readily incorporated into the reaction site and the formation of **C** is largely enhanced as a consequence. It is noteworthy that the attack of the hydrophobic indole on species **B** (β-replacement) becomes more favorable relative to the hydrolysis of **B** (β-elimination) due to significant elimination of water molecules from the reaction site (refer to Table 6). On the other hand, the hydrolysis of species **C** seems to be unfavorable in the bilayer phase because of the hydrophobic field effect. However, such disadvantage can be partly overcome by a general acid-base catalysis of the imidazolyl group of the lipid,[13-16] which is placed in the proximity site of the coenzyme moiety in the intramembrane domain.[12,13] The imidazolyl group may also catalyze the reaction steps, **A** to **B** and **B** to **C**, as a general acid or base, in a manner similar to that postulated for the enzymatic reactions.[22]

Addition of metal ions, such as bivalent copper and zinc, and tervalent aluminum and gallium, resulted in enhancement of the β-replacement of Ser with indole (Table 2). The coordination of such metal ions to the Schiff-base species must generate electron-withdrawing and template effects in favor of formation of the intermediate species, **A**, **B**, and **C**, and enhances the hydrolysis of **C** to afford Trp (refer to **A'**, **B'**, and **C'**)



A' **B'** **C'**

In the PL^+2C_{16}-$N^+C_5His2C_{16}$ system, the β-replacement was somewhat more favored under acidic pH conditions rather than that carried out under basic conditions regardless of the participation of copper(II) ions (Table 3). This implies that the acid-catalyzed reaction steps, elimination of the β-hydroxyl group of the Ser residue in **A** and hydrolysis of **C**, are concerned with determination of the overall rate of the β-replacement reaction. In the light of our previous kinetic analysis focused on the isomerization process between an aldimine Schiff-base and the corresponding ketimine Schiff-base in the transamination reaction,[13] the protonation at the α-position of the Trp residue in the carbanion Schiff-base intermediate to afford **C** is not referred to the rate-determining step.

When PL^+2C_{16} was replaced by $PL2C_{16}$ in the $N^+C_5His2C_{16}$ vesicle in the absence of metal ions, the catalytic efficiency of the latter in the β-replacement was comparable to that of the former at pH 5.0 but much depressed at pH 9.5 (Table 3). Since the pK_a value for the pyridyl nitrogen of the Schiff-base, derived from $PL2C_{16}$ and α-amino acid, in the vesicle presumably lies in a 6—7 range,[15,25] the pyridyl nitrogen of $PL2C_{16}$ is not protonated at pH 9.5 and a positive charge on the pyridyl nitrogen of PL^+2C_{16} acts to promote the β-replacement reaction as an electron sink.

The vesicular catalyst composed of PL^+2C_{16} and $N^+C_5His2C_{16}$ as an artificial tryptophan synthase exercised substrate selectivity toward indoles but not toward serine analogues (Tables 4 and 5). Thus, it became clear that the formation of aldimine Schiff-base **A** is not referred to the rate-determining step. In addition, the pH-effect in reactivity of benzyl L-serinate is similar to that of L-Ser as mentioned above. On the other hand, marked selectivity for indole derivatives primarily reflects differences in their nucleophilicity. The substrate selectivity of the present artificial tryptophan synthase is much different from that of the natural tryptophanase. It must be noted that the artificial enzyme is effective for the formation of 5-methoxytryptophan that can not be produced by the natural enzyme.

In conclusion, the present vesicular catalyst composed of PL^+2C_{16}, $N^+C_5His2C_{16}$, and copper(II) ions

behaves effectively as an artificial tryptophan synthase toward substrate couples of serine and indole analogues, showing high reaction selectivity (Table 6). On the other hand, the same catalyst can be used as an artificial transaminase effective for couples of hydrophobic α-amino acids and hydrophilic α-keto acids as substrates. The catalytic sites of tryptophan synthase and tryptophanase seem to exercise molecular recognition toward substrate species based on their sizes. On the other hand, the present artificial holoenzyme does not perform size-selective molecular discrimination due to softness of the intramembrane domain. However, a novel feature of molecular recognition, which reflects nucleophilicity of the second substrate (an indole derivative), is now realized. We are aiming at developing artificial holoenzymes capable of performing novel substrate specificity and reaction selectivity which have not been acknowledged by naturally occurring holoenzymes.

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