CHEMISTRY LETTERS, pp. 1765-1768, 1990.

Enantioselective Catalysis by Artificial Tryptophan Synthase Formed with Functionalized Bilayer Membrane

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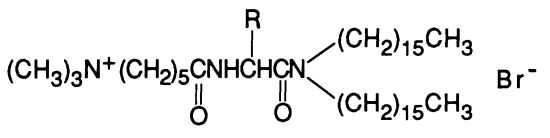
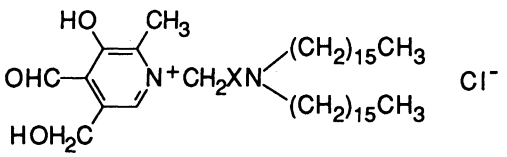
A functionalized bilayer membrane, composed of a cationic peptide lipid having a L-histidyl residue, a hydrophobic pyridoxal derivative, and copper(II) ions, catalyzed the β-replacement reaction of serine with indole to afford tryptophan in a significant enantiomeric excess of the D-isomer.

Recently, we have developed artificial enzymes having vitamin B_6 activity by employing synthetic bilayer membranes.[1-5] The bilayer vesicle composed of a synthetic peptide lipid, *N*,*N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5His2C_{16}$), and a hydrophobic vitamin B_6 derivative, 1-[(dihexadecylcarbamoyl)methyl]-4-formyl-3-hydroxy-5-hydroxymethyl-2-methylpyridinium chloride ($PL^+C_1CON2C_{16}$), exhibits efficient catalytic activity in the transamination reaction of α-amino acids with α-keto acids upon addition of copper(II) ions in aqueous media under mild conditions, showing high substrate selectivity.[1] The identical vesicular catalyst behaves as an artificial tryptophan synthase which mediates the β-replacement reactions of L-serine (L-Ser) with indoles to afford the corresponding tryptophan derivatives (refer to Eq. 1).[4,5] In the present



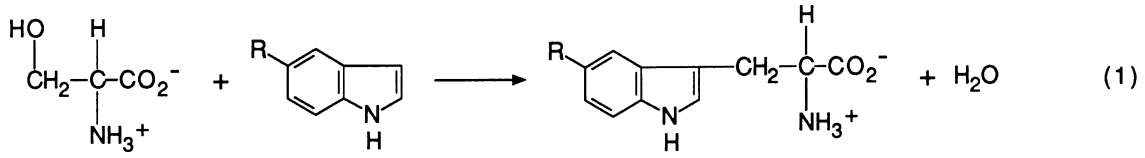
$N^+C_5His2C_{16}$: R = CH_2-imidazole

$N^+C_5Ala2C_{16}$: R = CH_3



$PL^+C_1CON2C_{16}$: X = CO

$PL^+C_2N2C_{16}$: X = CH_2



study, we clarified that an artificial tryptophan synthase formed with $N^+C_5His2C_{16}$ and a hydrophobic pyridoxal derivative, 1-[2-(dihexadecylamino)ethyl]-4-formyl-3-hydroxy-5-hydroxymethyl-2-methylpyridinium chloride ($PL^+C_2N2C_{16}$),[6] and copper(II) ions catalyzes the β-replacement reaction of serine with indole to afford tryptophan in a significant enantiomeric excess of the D-isomer.

The β-replacement reaction of serine with indole was studied in an aqueous acetate buffer (25 mmol dm^{-3}, μ 0.04 with KCl) at pH 5.0 and 30.0 °C, in the presence of molecular aggregates formed with $PL^+C_2N2C_{16}$ and each of the following amphiphiles; hexadecyltrimethylammonium bromide (CTAB), *N,N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{16}$), and $N^+C_5His2C_{16}$. The highest catalytic activity for the β-replacement reaction was achieved by the vesicular system composed of $N^+C_5His2C_{16}$, $PL^+C_2N2C_{16}$, and copper(II) ions among these aggregates, in analogy with our previous work carried out by employing $PL^+C_1CON2C_{16}$ in place of $PL^+C_2N2C_{16}$.[4,5] Selectivity toward β-replacement and β-elimination reactions for L-Ser, as exercised by the molecular aggregates in the presence of copper(II) ions, is listed in Table 1. Since the overall reactivity of the β-replacement and β-elimination reactions is comparable to each other among these catalyst systems, a difference in aggregation mode, micelles or bilayer vesicles, does not give out much influence in the initial reaction steps; formation of the aldimine Schiff-base chelate (**A** in Scheme 1) from $PL^+C_2N2C_{16}$, L-Ser, and copper(II) ions, and the subsequent transformation of **A** into the α,β-eliminated intermediate (**B** in Scheme 1). Thus, the bilayer aggregates formed with the synthetic peptide lipids provide a more favorable reaction site for an attack of the indole molecule on **B**, in comparison with the CTAB micelle.

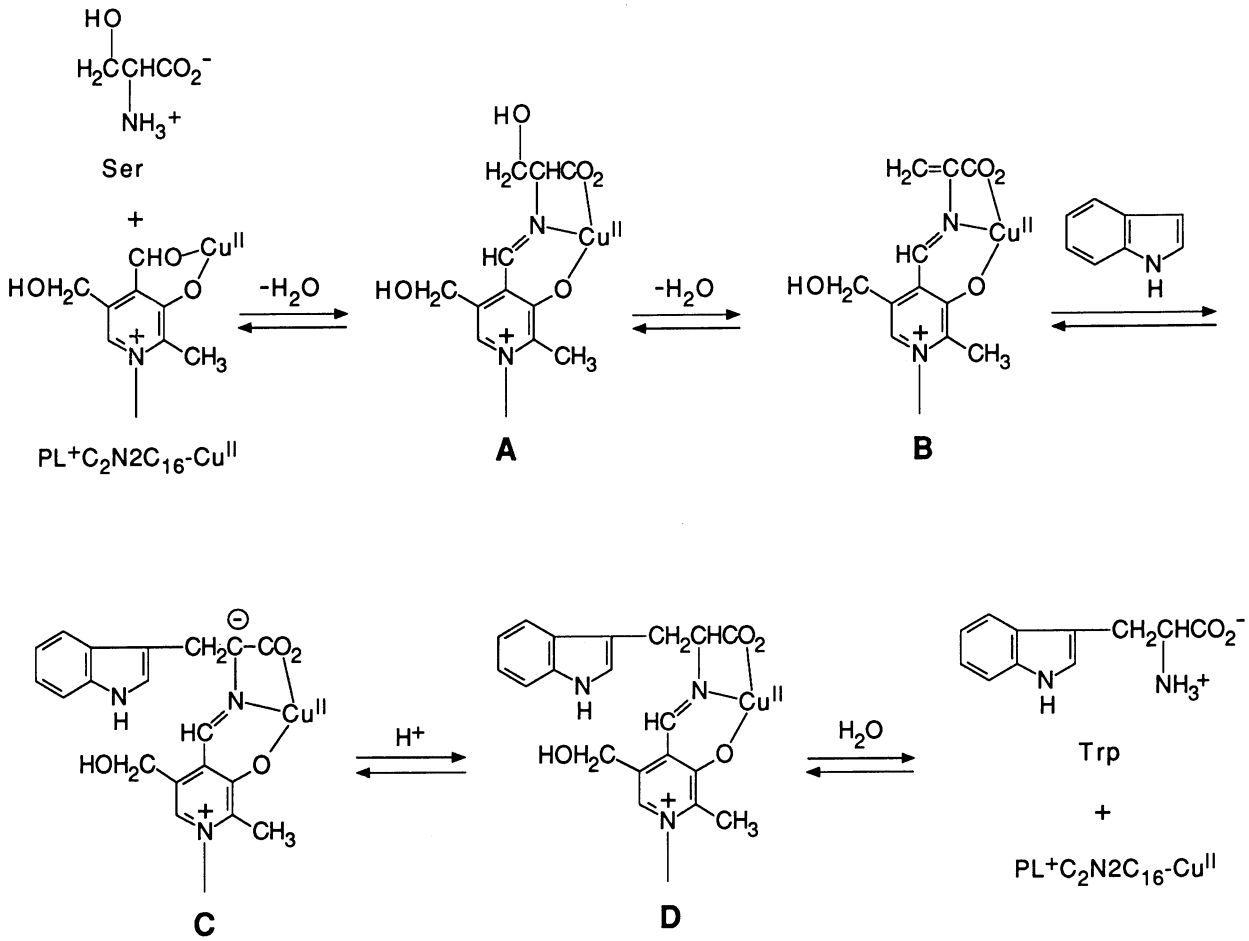
It is noteworthy that the $N^+C_5His2C_{16} - PL^+C_2N2C_{16} - Cu(II)$ system shows marked enantioselectivity in the β-replacement reaction. As listed in Table 2, the formation of D-tryptophan prevails over that of the corresponding L-form in 50–55% e.e. regardless of chirality of the substrate, serine. On the other hand, any detectable enantioselectivity was not observed when the $N^+C_5Ala2C_{16}$ vesicule and the CTAB micelle were used in place of the $N^+C_5His2C_{16}$ vesicle. Such results mean that the imidazolyl group of the L-histidyl residue introduced covalently into the peptide lipid exercises stereospecific acid catalysis in the protonation to the prochiral carbanion intermediate (**C** in Scheme 1) to afford the aldimine Schiff-base of $PL^+C_2N2C_{16}$ with tryptophan (**D** in Scheme 1).

In conclusion, it became apparent that the functionalized bilayer vesicle formed

Table 1. Reaction Selectivity Exhibited by Artificial Enzymes Formed with $PL^+C_2N2C_{16}$, Amphiphiles, and Copper(II) Ions at 30.0 ± 0.1 °C[a)]

Amphiphile	Relative yield[b)]	
	β-Replacement[c)]	β-Elimination[d)]
CTAB	1.0[e)]	6.9
$N^+C_5Ala2C_{16}$	3.6	2.8
$N^+C_5His2C_{16}$	5.1	2.3

a) In an aqueous acetate buffer (25 mmol dm^{-3}, μ 0.04 with KCl) at pH 5.0. Concentrations in mmol dm^{-3}: L-Ser, 5.0; indole, 5.0; $PL^+C_2N2C_{16}$, 0.05; CTAB, 3.0; $N^+C_5Ala2C_{16}$ and $N^+C_5His2C_{16}$, 1.0; $Cu(ClO_4)_2$, 0.05. b) Evaluated after incubation for 200 h. c) β-Replacement product, tryptophan, was analyzed by HPLC on a column of TSK gel ODS-120T. d) β-Elimination product, pyruvate, was analyzed by HPLC after its conversion into the fluorescent 3-methyl-2-quinoxalinol by reaction with *o*-phenylenediamine (Ref. 7). e) Yield, 5.1 x 10^{-6} mol dm^{-3}.



Scheme 1.

Table 2. Enantioselectivity Exhibited by Artificial Enzyme Formed with $PL^+C_2N2C_{16}$, $N^+C_5His2C_{16}$, and Copper(II) Ions at 30.0 ± 0.1 °C[a)]

Chirality of serine	Total yield of tryptophan/mol dm^{-3} [b)]	e.e. of D-isomer/%[c)]
L	3.0×10^{-5}	50
D	3.0×10^{-5}	55
DL	3.0×10^{-5}	51

a) In an aqueous acetate buffer (25 mmol dm^{-3}, μ 0.04 with KCl) at pH 5.0. Concentrations in mmol dm^{-3}: L-Ser, 5.0; indole, 5.0; $PL^+C_2N2C_{16}$, 0.05; $N^+C_5His2C_{16}$; 1.0; $Cu(ClO_4)_2$, 0.05. b) Evaluated after incubation for 200 h. c) 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 an eluant.

with $N^+C_5His2C_{16}$, $PL^+C_2N2C_{16}$, and copper(II) ions effectively catalyzed the β-replacement reaction of serine with indole to afford tryptophan with chiral priority of its D-isomer. Detailed mechanistic analysis of the enantioselective catalysis is now in progress in our laboratory.

References

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6) $PL^+C_2N2C_{16}$ was prepared by the reaction of pyridoxal monomethylacetal with *N,N*-dihexadecyl-2-iodoethylamine, followed by hydrolysis to give a brown solid (the hemiacetal form); mp 145 °C (decomp); 500 MHz ^1H NMR ($CDCl_3$) δ= 0.88 [6H, t, $CH_2(CH_2)_{14}C\underline{H}_3$], 1.25 [56H, s, $CH_2(C\underline{H}_2)_{14}CH_3$], 2.86 [3H, s, CH_3 on pyridine ring], 3.22 [4H, m, $C\underline{H}_2(CH_2)_{14}CH_3$], 3.49 [2H, m, CH_2N], 3.80 [2H, m, N^+CH_2 on pyridine ring], 5.10 [2H, dd, CH_2O on pyridine ring], 6.70 [1H, s, C<u>H</u>OH], 8.89 [1H, s, H on pyridine ring]. Found: C, 65.90; H, 11.00; N, 3.62%. Calcd for $C_{42}H_{79}ClO_3N_2 \cdot HCl \cdot 2H_2O$: C, 65.68; H,11.02; N, 3.65%.
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(Received July 19, 1990)