

Enantioselective Catalysis by Artificial Tryptophan Synthase Formed with Functionalized Bilayer Membrane

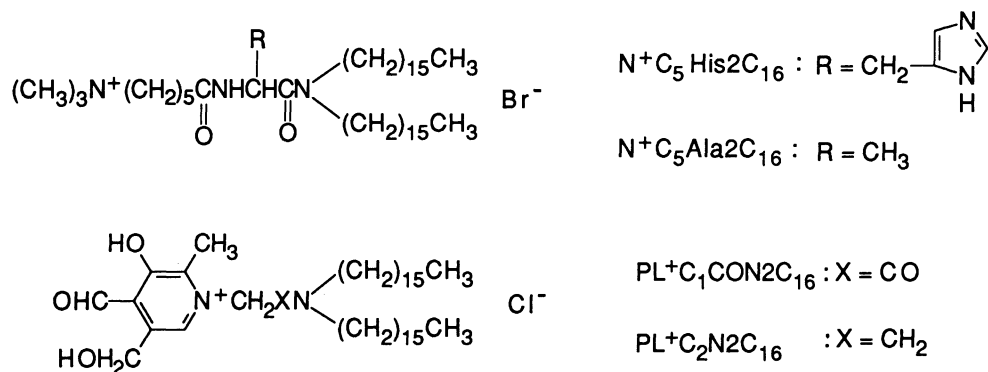
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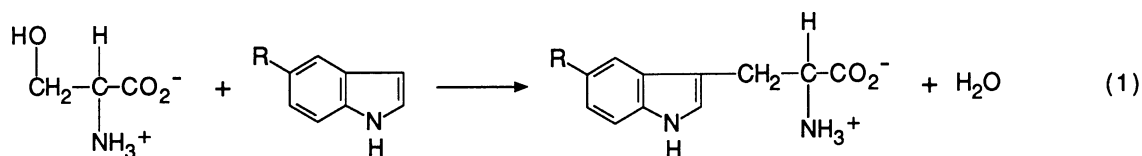
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A functionalized bilayer membrane, composed of a cationic peptide lipid having a L-histidyl residue, a hydrophobic pyridoxal derivative, and copper(II) ions, catalyzed the β -replacement reaction of serine with indole to afford tryptophan in a significant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

Recently, we have developed artificial enzymes having vitamin B₆ activity by employing synthetic bilayer membranes.¹⁻⁵⁾ The bilayer vesicle composed of a synthetic peptide lipid, *N,N*-dihexadecyl-*N* α -[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5\text{His}2C_{16}$), and a hydrophobic vitamin B₆ derivative, 1-[(dihexadecylcarbamoyl)methyl]-4-formyl-3-hydroxy-5-hydroxymethyl-2-methylpyridinium chloride ($PL^+C_1\text{CON}2C_{16}$), 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





study, we clarified that an artificial tryptophan synthase formed with $\text{N}^+\text{C}_5\text{His}2\text{C}_{16}$ and a hydrophobic pyridoxal derivative, 1-[2-(dihexadecylamino)ethyl]-4-formyl-3-hydroxy-5-hydroxymethyl-2-methylpyridinium chloride ($\text{PL}^+\text{C}_2\text{N}2\text{C}_{16}$),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 $\text{PL}^+\text{C}_2\text{N}2\text{C}_{16}$ and each of the following amphiphiles; hexadecyltrimethylammonium bromide (CTAB), *N,N*-dihexadecyl-*N* $^\alpha$ -[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($\text{N}^+\text{C}_5\text{Ala}2\text{C}_{16}$), and $\text{N}^+\text{C}_5\text{His}2\text{C}_{16}$. The highest catalytic activity for the β -replacement reaction was achieved by the vesicular system composed of $\text{N}^+\text{C}_5\text{His}2\text{C}_{16}$, $\text{PL}^+\text{C}_2\text{N}2\text{C}_{16}$, and copper(II) ions among these aggregates, in analogy with our previous work carried out by employing $\text{PL}^+\text{C}_1\text{CON}2\text{C}_{16}$ in place of $\text{PL}^+\text{C}_2\text{N}2\text{C}_{16}$.^{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 $\text{PL}^+\text{C}_2\text{N}2\text{C}_{16}$, 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.

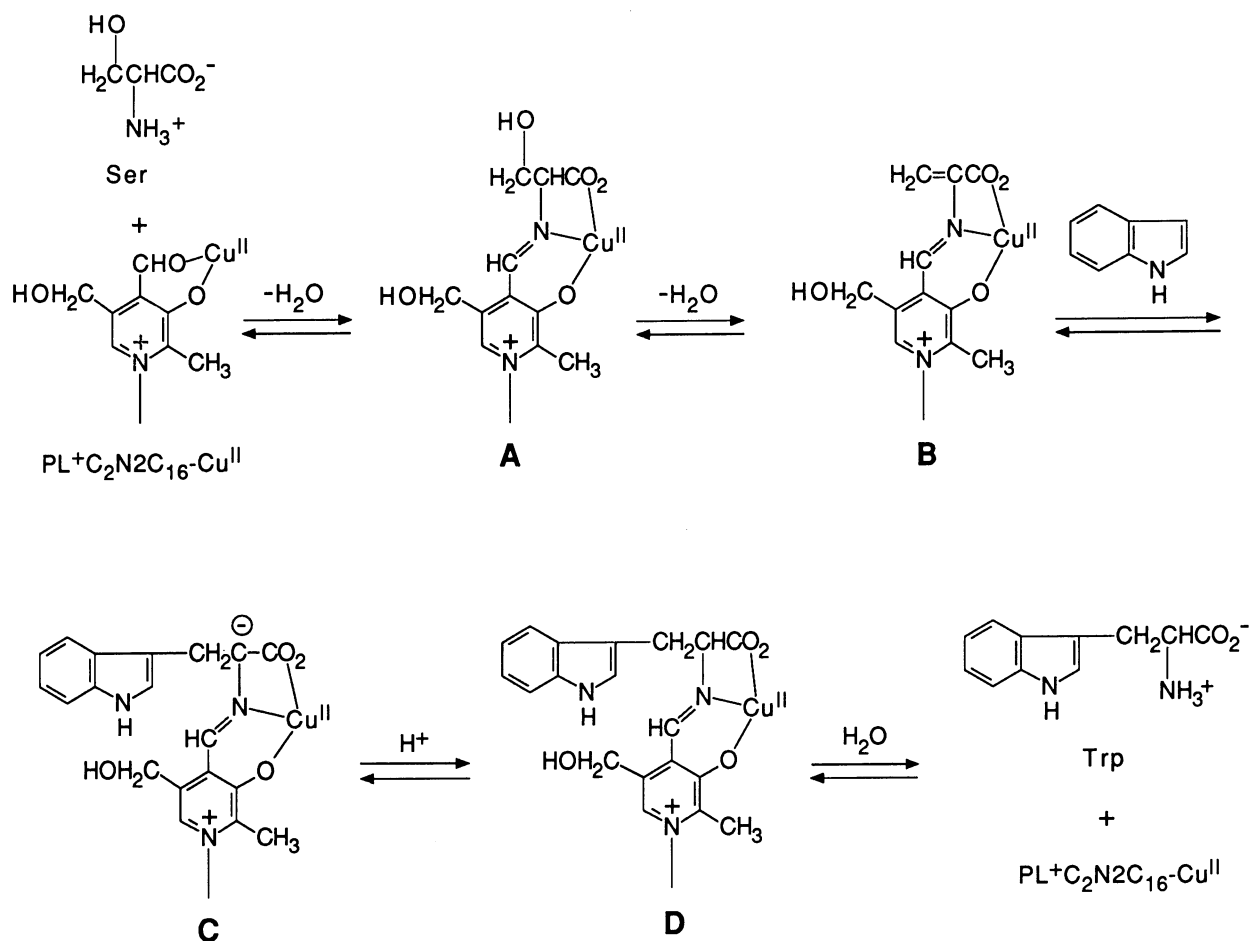
It is noteworthy that the $\text{N}^+\text{C}_5\text{His}2\text{C}_{16} - \text{PL}^+\text{C}_2\text{N}2\text{C}_{16} - \text{Cu(II)}$ system shows marked enantioselectivity in the β -replacement reaction. As listed in Table 2, the formation of D-tryptophan prevails over that of the corresponding L-form in 50–55% e.e. regardless of chirality of the substrate, serine. On the other hand, any detectable enantioselectivity was not observed when the $\text{N}^+\text{C}_5\text{Ala}2\text{C}_{16}$ vesicle and the CTAB micelle were used in place of the $\text{N}^+\text{C}_5\text{His}2\text{C}_{16}$ 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 $\text{PL}^+\text{C}_2\text{N}2\text{C}_{16}$ 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+C₂N₂C₁₆, 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 ₅ Ala ₂ C ₁₆	3.6	2.8
N ⁺ C ₅ His ₂ C 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+C₂N₂C₁₆, 0.05; CTAB, 3.0; N⁺C₅Ala₂C₁₆ and N⁺C₅His₂C₁₆, 1.0; Cu(ClO₄)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+C₂N₂C₁₆, N⁺C₅His₂C₁₆, 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 x 10 ⁻⁵	50
D	3.0 x 10 ⁻⁵	55
DL	3.0 x 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+C₂N₂C₁₆, 0.05; N⁺C₅His₂C₁₆; 1.0; Cu(ClO₄)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⁺C₅His₂C₁₆, PL+C₂N₂C₁₆, 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+C₂N₂C₁₆ 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 (CDCl₃) δ= 0.88 [6H, t, CH₂(CH₂)₁₄CH₃], 1.25 [56H, s, CH₂(CH₂)₁₄CH₃], 2.86 [3H, s, CH₃ on pyridine ring], 3.22 [4H, m, CH₂(CH₂)₁₄CH₃], 3.49 [2H, m, CH₂N], 3.80 [2H, m, N⁺CH₂ on pyridine ring], 5.10 [2H, dd, CH₂O on pyridine ring], 6.70 [1H, s, CHOH], 8.89 [1H, s, H on pyridine ring]. Found: C, 65.90; H, 11.00; N, 3.62%. Calcd for C₄₂H₇₉ClO₃N₂·HCl·2H₂O: C, 65.68; H, 11.02; N, 3.65%.
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