Transamination Reaction of Hydrophobic Pyridoxal with an α-Amino Acid in Functionalized Bilayer Vesicles: Co-operative Catalysis by the Imidazolyl Group and Copper(II) Ions

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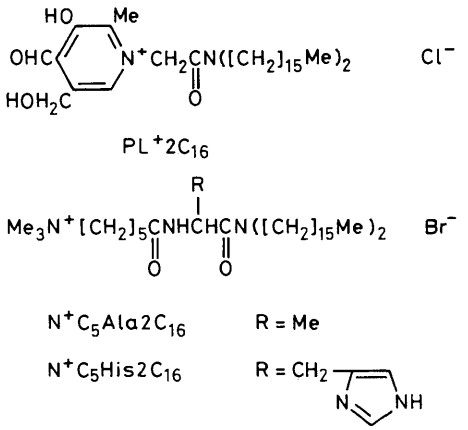
1-(*N,N*-Dihexadecylcarbamoylmethyl)-2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridinium chloride (PL^+2C_{16}) undergoes a transamination reaction with L-phenylalanine in single-walled bilayer vesicles formed from two different peptide lipids ($N^+C_5Ala2C_{16}$ and $N^+C_5His2C_{16}$); co-ordination of copper(II) ion to the Schiff-base intermediate results in a marked rate acceleration.

The aim of our research is to create holoenzyme models to simulate some functional aspects of vitamin B_6-dependent enzymes. We have based our work on the following information: (i) that Schiff-base formation between pyridoxal and a hydrophobic α-amino acid is much enhanced in molecular aggregates such as micelles and vesicles, so that the overall transamination reaction is considerably accelerated;[1—3] (ii) a positive charge on the pyridyl nitrogen of pyridoxal provides an electron sink which promotes the α-hydrogen abstraction from an aldimine Schiff-base;[2,4] (iii) when the pyridoxal moiety is fixed in the so-called hydrogen-belt domain formed by the histidyl residues interposed between the hydrophobic and hydrophilic zones within the vesicles, the imidazolyl group induces an intramolecular prototropic shift to give the corresponding ketimine Schiff-base and accelerates the overall transamination reaction.[1,3] We report here on the transamination reaction of PL^+2C_{16}† with L-phenylalanine (L-Phe) (Scheme 1) in single-walled bilayer vesicles formed from the synthetic peptide lipids, $N^+C_5Ala2C_{16}$[6] and $N^+C_5His2C_{16}$.‡

Aqueous dispersions of either of the peptide lipids (1.0×10^{-3} mol dm^{-3}) containing PL^+2C_{16} (5.0×10^{-5} mol dm^{-3}) in an aqueous carbonate buffer (2.0×10^{-2} mol dm^{-3}, pH 9.9, μ 0.10 M with KCl) were sonicated for 1 min with a probe-type sonicator at 30 W to give single-walled bilayer vesicles. The transamination reaction in the vesicles was followed spectrophotometrically at 30.0 °C. When PL^+2C_{16} is incorporated into the respective vesicles formed from $N^+C_5Ala2C_{16}$ and $N^+C_5His2C_{16}$, these vesicles provide for the pyridoxal moiety a microenvironment equivalent to propan-1-ol in polarity [$E_T(30)$ = 50.7]. The extent of formation of an aldimine Schiff-base of PL^+2C_{16} with an α-amino acid in the vesicles is dependent on the hydrophobicity of the α-amino acid (equilibrium constants K_{SB} at pH 9.9 in the $N^+C_5Ala2C_{16}$ vesicle: L-Ala *ca.* 10, L-Leu 560, L-Phe 1700 mol^{-1} dm^3).



As observed for the transamination reaction of pyridoxal-5′-phosphate with *N*-dodecyl-L-alaninamide in vesicles,[1] the reaction proceeded through the fast equilibrated formation of an aldimine Schiff-base [(A) in Scheme 1] followed by much slower conversion into the pyridoxamine derivative (PM^+2C_{16}) and β-phenylpyruvate (KA).§ The rate-determining step in the overall reaction is the isomerization of the aldimine Schiff-base [(A) in Scheme 1] to the corresponding ketimine [(C) in Scheme 1] since the accumulation of the ketimine Schiff-base was not observed to any detectable extent during the reaction. The pH-dependent equilibrium constants (K_{SB}) for the formation of (A) and the rate constants (k) for the isomerization, as defined in equation (1) [(AA) for L-Phe], are listed in Table 1.¶

$$PL^+2C_{16} + (AA) \overset{K_{SB}}{\rightleftharpoons} (A) \overset{k}{\rightarrow} PM^+2C_{16} + (KA) \quad (1)$$

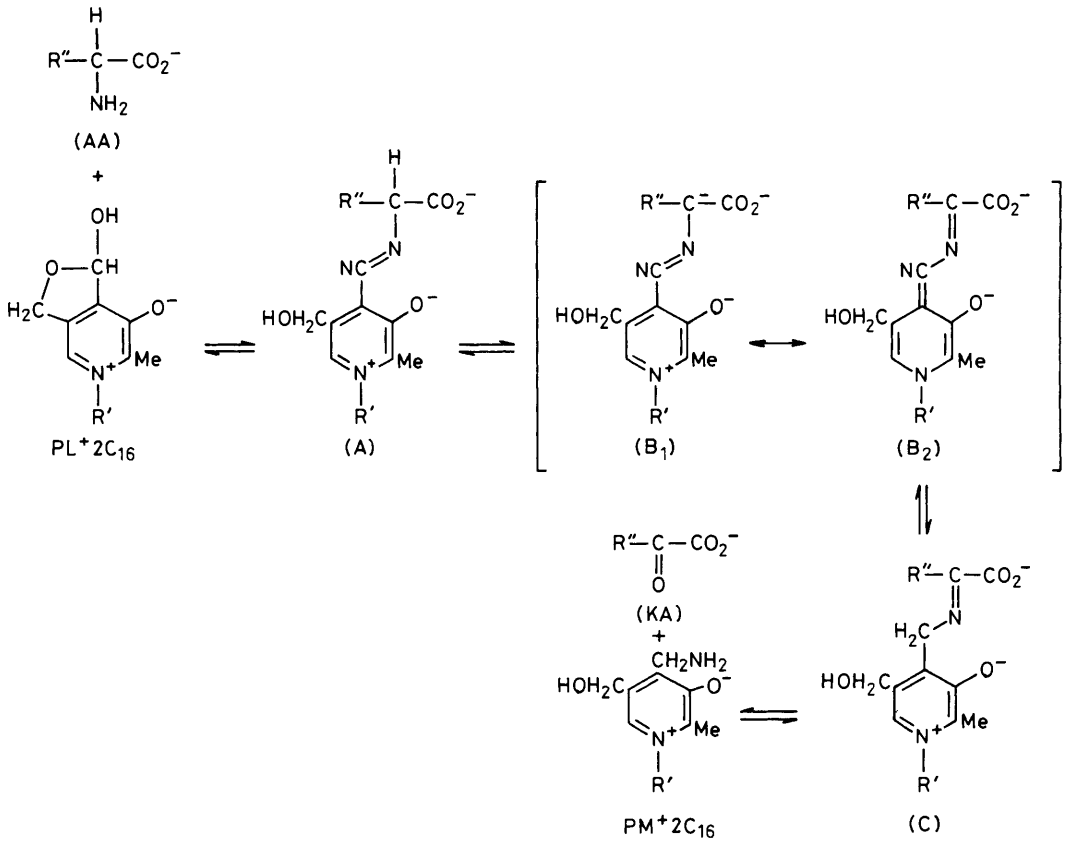
The K_{SB} value for 1,2-dimethyl-3-hydroxy-4-formyl-5-hydroxymethylpyridinium chloride[5] with L-Phe is extremely low (10 mol^{-1} dm^3) in aqueous carbonate buffer (2.0×10^{-2} mol dm^{-3}, pH 9.9, μ 0.10 M with KCl) at 30.0 °C. The transamination was followed spectrophotometrically in the presence of a 1000-fold excess of L-Phe (5.0×10^{-2} mol dm^{-3}) over the pyridoxal derivative; $k_{obs} = 2 \times 10^{-6}$, $k = 6 \times 10^{-6}$ s^{-1}. Since this k value is comparable with those obtained for the vesicular systems, the overall rate acceleration in the vesicles originates predominantly from the enhancement of Schiff-base formation.

† PL^+2C_{16} was prepared by the reaction of pyridoxal monomethylacetal (ref. 5) with *N,N*-dihexadecyl-2-iodoacetamide, followed by hydrolysis to give a yellow solid, characterised by elemental analysis and n.m.r. spectroscopy.

‡ $N^+C_5His2C_{16}$ was prepared as described previously (ref. 1) as a colourless glassy solid, characterised by elemental analysis and n.m.r. spectroscopy.

§ PL^+2C_{16} was separated from the reaction mixture by extraction with chloroform. The extracted PL^+2C_{16} and the (KA) remaining in the reaction medium were separately converted into the corresponding 2,4-dinitrophenylhydrazones and their amounts were quantitatively evaluated by spectrophotometric means. PM^+2C_{16} was extracted with toluene from the reaction mixture and then treated with 2,4,6-trinitrobenzenesulphonate before identification and quantitative evaluation by spectrophotometry: A. P. H. Phan, T. T. Ngo, and H. M. Lenhoff, *Appl. Biochem. Biotech.*, 1983, **8**, 127. When the transamination was completed both in the presence and absence of copper(II) ions, PL^+2C_{16} was not detected in the reaction mixture and PM^+2C_{16} and (KA) were obtained quantitatively.

¶ The apparent first-order rate constant (k_{obs}) for the disappearance of the aldimine Schiff-base was calculated from the initial rate of absorbance decay at 393 nm and is correlated with K_{SB} and k by the equation: $k_{obs} = kK_{SB}[AA]_0/(K_{SB}[AA]_0 + 1)$; where $[AA]_0$ denotes the initial concentration of the amino acid (ref. 2).



$R' = CH_2C(O)N([CH_2]_{15}Me)_2$

$R'' = CH_2Ph$

Scheme 1

Addition of copper(II) ions to the reaction system in equation (1) caused a drastic change in reaction behaviour. When copper(II) perchlorate (2-fold molar excess over PL^+2C_{16}) was added to the equilibrium mixture of the aldimine Schiff-base, PL^+2C_{16}, and L-Phe, obtained in the initial fast step, the spectral changes shown in (3) in Figure 1 were observed in both vesicular systems. Two different Cu^{II} complex species, which have absorption maxima at 523 and *ca.* 390 nm and are mutually in equilibrium, were formed within 5 s,** and the former species underwent reaction to give PM^+2C_{16} and (KA).

The 523 nm band that appeared upon addition of copper(II) ions is due to the copper(II) complex of the carbanion

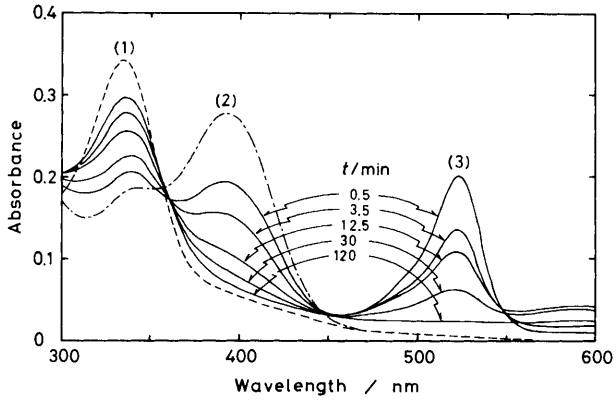


Figure 1. Absorption spectral changes for the Cu^{II}-catalysed transamination reaction of PL^+2C_{16} (5.0×10^{-5} mol dm^{-3}) with L-Phe (5.0×10^{-3} mol dm^{-3}) in the presence of $N^+C_5Ala2C_{16}$ (1.0×10^{-3} mol dm^{-3}) in aqueous carbonate buffer (2.0×10^{-2} mol dm^{-3}, pH 9.9, μ 0.10 M with KCl) at 30.0 °C: (1) (– – –) PL^+2C_{16} alone; (2) (— · — · —) equilibrium mixture of PL^+2C_{16}, L-Phe, and (A) (Scheme 1) without Cu^{II}; (3) (——) Cu^{II} complex species formed upon addition of $Cu(ClO_4)_2$ (1.0×10^{-4} mol dm^{-3}) to (2). The curves in (3) are distinguished by figures which give the reaction period (t) in min after addition of copper(II) ions.

** The absorption maximum at *ca.* 390 nm for the Cu^{II}–(A) complex, which was formed instantaneously upon addition of copper(II) perchlorate to (A), is very close to that for (A). PL^+2C_{16} (5.0×10^{-5} mol dm^{-3}) underwent reaction with L-Phe (5.0×10^{-3} mol dm^{-3}) to afford the corresponding aldimine Schiff-base at *ca.* 30% conversion at pH 7.6 in the $N^+C_5Ala2C_{16}$ vesicle. The absorption band at *ca.* 400 nm increased instantaneously upon addition of copper(II) perchlorate (1.0×10^{-4} mol dm^{-3}) without showing any significant band shift. The copper(II) complex of the aldimine Schiff-base formed from 2-methyl-3-hydroxy-4-formyl-5-dodecylthiomethylpyridine and L-alanine shows an absorption maximum at *ca.* 390 nm in vesicular systems as reported previously (ref. 3).

Table 1. Kinetic parameters for the reaction of $PL^{+}2C_{16}$ with L-Phe in single-walled vesicles at 30.0 °C.[a]

	Metal-free[b]			Cu[II]-catalysed[c]
Peptide lipid	$k_{obs} \times 10^{6}/s^{-1}$	$K_{SB}/mol^{-1}\,dm^{3}$	$k \times 10^{6}/s^{-1}$	$k_{obs}^{Cu} \times 10^{3}/s^{-1}$
$N^{+}C_5His2C_{16}$	6.5	830	8.1	23
$N^{+}C_5Ala2C_{16}$	1.5	1700	1.6	4.3

[a] Initial concentrations in mol dm^{-3}: $PL^{+}2C_{16}$, 5.0×10^{-5}; L-Phe, 5.0×10^{-3}; peptide lipids, 1.0×10^{-3}. Medium: 2.0×10^{-2} mol dm^{-3} aqueous carbonate buffer (pH 9.9) containing 0.10 mol dm^{-3} KCl. [b] Ethylenediaminetetra-acetic acid (1.0×10^{-4} mol dm^{-3}) was added. [c] $Cu(ClO_4)_2$ (1.0×10^{-4} mol dm^{-3}) was added immediately after aldimine Schiff-base formation.

intermediate [(B_1) and (B_2) in Scheme 1] derived by α-hydrogen abstraction from the Cu^{II}–aldimine Schiff-base chelate. This is based on the following observations. (i) When DL-phenylglycine was substituted for L-Phe, the corresponding absorption band appeared at 533 nm (red shift by 10 nm), whereas the combination of $PL^{+}2C_{16}$ and dodecylamine did not show such a spectral change upon addition of copper(II) ions. (ii) Addition of 5,5′-dithiobis(2-nitrobenzoic acid) which reacts with carbanions, to the copper(II)-containing system gave instantaneously a new absorption band at *ca.* 420 nm, a characteristic band for the 3-carboxy-4-nitrothiophenolate anion, and caused the disappearance of the 523 nm band. (iii) When methyl L-phenylalaninate was used as a substrate, a species with an absorption maximum at 502 nm, a characteristic band for the carbanion intermediate, was formed even in the absence of copper(II) ions. Upon addition of copper(II) perchlorate, the band was shifted to 523 nm. (iv) Such carbanion intermediates have been detected in enzymic and nonenzymic systems,[7] although there is very limited formation of the carbanion intermediate in nonenzymic systems containing α-amino acids.‖

In conclusion, the co-ordination of copper(II) ion to the aldimine Schiff-base significantly enhanced deprotonation at the α-carbon atom so that the rate-determining step was changed from (A)→(C) to (B_1), (B_2)→(C) (Scheme 1) and the isomerization was accelerated dramatically (Table 1). The higher reactivity in the $N^{+}C_5His2C_{16}$ vesicle relative to that in the $N^{+}C_5Ala2C_{16}$ vesicle must originate from the catalytic function of the imidazolyl group. The single-walled vesicles formed from peptide lipids can provide extremely effective reaction sites for acceleration of transamination reactions.

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‖ The carbanion intermediate was not detected during the reaction of 1,2-dimethyl-3-hydroxy-4-formyl-5-hydroxymethylpyridinium chloride with L-Phe in the presence of copper(II) ion in aqueous carbonate buffer at pH 9.9, μ 0.10 M, and 30.0 °C.

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