Functionalised Bilayer Vesicle as a Catalyst for Transamination: Artificial Transaminase

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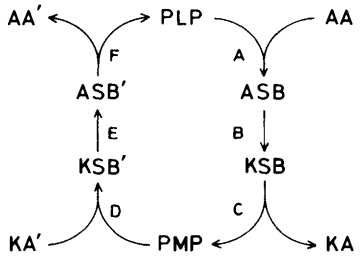
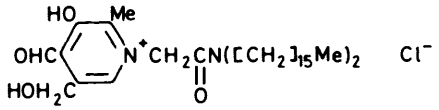
The non-enzymic transamination reaction of α-amino acids with α-keto acids was investigated in aqueous media at 30.0 °C. The functionalised single-walled co-vesicle composed of a synthetic peptide lipid, *NN*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide, and a hydrophobic pyridoxal derivative, 1-(*NN*-dihexadecylcarbamoylmethyl)-2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridinium chloride, effectively catalysed amino-group transfer from L-phenylalanine to pyruvic acid in the presence of copper(II) ions, showing turnover behaviour. The catalytic activity of the vesicular system was much higher than those of 1,2-dimethyl-3-hydroxy-4-formyl-5-hydroxymethylpyridinium chloride and pyridoxal examined in aqueous media containing copper(II) ions. The rate-determining step involved in the catalytic cycle performed with the vesicular catalyst is primarily assigned to the product-releasing process, the hydrolysis of the copper(II) chelate of the aldimine Schiff's base to afford alanine.

Vitamin B_6-dependent transaminases catalyse the amino-group transfer from α-amino acids (AA) to α-keto acids (KA′) and take part in amino-acid metabolism.[1] It is well known that the catalytic cycle is completed by the sequential reactions in the enzymic system shown in the Scheme: (i) formation of the aldimine Schiff's base (ASB) of AA with the coenzyme, pyridoxal 5′-phosphate (PLP), which is tightly bound to the apoprotein (step A); (ii) isomerization of ASB to the ketimine Schiff's base (KSB) (step B); (iii) hydrolysis of KSB to give an α-keto acid (KA) and pyridoxamine 5′-phosphate (PMP) (step C); (iv) reversal of this sequence of reactions, starting with PMP and another α-keto acid (KA′), to afford PLP and the corresponding α-amino acid (AA′) *via* formation of the ketimine and aldimine Schiff's bases (KSB′ and ASB′, respectively) (steps D ⟶ E ⟶ F). Although the half-transamination reactions (A ⟶ B ⟶ C; D ⟶ E ⟶ F) proceed in non-enzymic systems,[2] such model reaction systems are much less efficient than the enzymic ones.

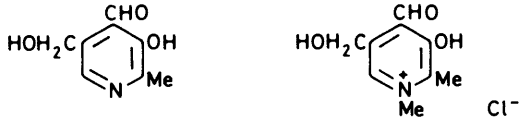
In order that an effective catalytic cycle is performed as shown in the Scheme, we set up a functionalised bilayer co-vesicle as an artificial transaminase. The system is constructed with a peptide lipid bearing the histidyl residue, *NN*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^{+}C_5His2C_{16}$), a pyridoxal analogue having a hydrophobic double-chain segment substituted on the pyridyl nitrogen, 1-(*NN*-dihexadecylcarbamoylmethyl)-2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridinium chloride (PL^{+}-$2C_{16}$), and copper(II) ions. The functionalised vesicle effectively catalyses the transamination of a hydrophobic α-amino acid, L-phenylalanine (L-Phe), with a hydrophilic α-keto acid, pyruvic acid, showing turnover behaviour. We are also to discuss the mechanistic aspect of the non-enzymic transamination reaction in comparison with the similar reactions carried out in the vesicle of *NN*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^{+}C_5Ala2C_{16}$) and the hexadecyltrimethylammonium bromide (CTAB) micelle. The catalytic reactivities of homogeneous systems containing pyridoxal (PL) and 1,2-dimethyl-3-hydroxy-4-formyl-5-hydroxymethylpyridinium chloride ($PL^{+}C_1$) were examined in the presence of copper(II) ions for comparison.



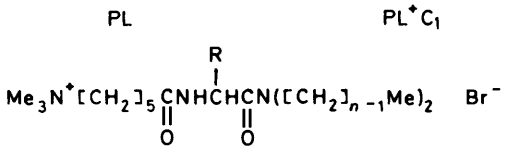
Scheme.



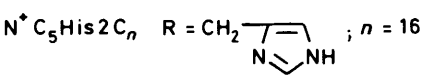
$PL^{+}2C_{16}$



PL $PL^{+}C_1$



$N^{+}C_5Ala2C_n$ R = Me; n = 14, 16



Results and Discussion

Molecular Basis for Designing the Artificial Enzyme.—Two critical requirements at least need to be fulfilled in order to set up an artificial enzyme which is effective for the transamination reaction between an α-amino acid and an α-keto acid: acceleration of the isomerization between the ketimine and aldimine Schiff's bases (steps B and E in the Scheme), since these processes often become the rate-determining steps in the non-enzymic reaction; and substrate selectivity in the Schiff's base formation processes (steps A and D in the Scheme).

In order to enhance the isomerization reactivity, we made three kinds of manipulation. First, the pyridyl nitrogen of the pyridoxal moiety was quaternized since a positive charge at this position is effective in providing an electron sink which promotes α-hydrogen abstraction from the aldimine Schiff's base [3,4] and favours the hydrogen abstraction at the C(4′) position of the ketimine Schiff's base. Second, the imidazolyl groups are buried in the intramembrane region as the histidyl residues of the lipid molecules, which constitute the so-called hydrogen-belt domain [5] interposed between the hydrophobic domain formed with aliphatic double-chains and the hydrophilic zone with ionic head groups. In order to attain the effective general acid–base catalysis by the imidazolyl group in the isomerization of the Schiff's bases,[6–8] tight anchoring of the coenzyme moiety in the hydrogen-belt domain is required.[9] A hydrophobic double-chain segment was introduced into the pyridoxal moiety so as to interact with the matrix membrane by a marked hydrophobic effect. Thirdly, the copper(II) ion was added to the reaction system, since this metal ion induces a significant co-ordination effect on the acceleration of the transamination reaction.[10] In fact, the transamination of PL^+2C_{16} with L-Phe was drastically accelerated in the presence of copper(II) ions and single-walled vesicles of $N^+C_5His2C_{16}$, relative to the reaction in an aqueous phase.[11] We have also clarified that the transamination of hydrophobic pyridoxamines, 2-methyl-3-hydroxy-4-aminomethyl-5-[(*NN*-dihexadecylamino)methyl]-pyridine ($PM2C_{16}$) and 2-methyl-3-hydroxy-4-aminomethyl-5-(dodecylthiomethyl)pyridine (PMC_{12}), with pyruvic acid in single-walled vesicles is effectively catalysed by copper(II) ions and the co-ordination-free imidazolyl group of membrane lipids.[9,12,13]

Amino acids are subjected to selective Schiff's base formation with hydrophobic pyridoxals in molecular aggregates such as micelles [4] and vesicles [11] which provide hydrophobic reaction sites in aqueous media. For instance, the formation constants for the aldimine Schiff's bases of PL^+2C_{16} with L-alanine (L-Ala) and L-Phe at pH 9.9 and 30.0 °C in the $N^+C_5Ala2C_{16}$ vesicle are *ca.* 10 and 1 700 $dm^3\ mol^{-1}$, respectively.[11] Even though a hydrophilic α-keto acid in anionic form, pyruvate, is concentrated in the electrostatic layer of cationic molecular aggregates, relatively large formation constants (K_{KSB}) were obtained for the ketimine Schiff's bases with hydrophobic pyridoxamines: the K_{KSB} value for PMC_{12} with pyruvate at pH 6.8 and 30.0 °C in the single-walled vesicle of *NN*-ditetradecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{14}$) is $6.4 \times 10^2\ dm^3\ mol^{-1}$, while that for pyridoxamine with pyruvate at pH 6.70 and 25 °C in an aqueous phosphate buffer is $1.15\ dm^3\ mol^{-1}$.[14] Accordingly, it seems that amino-group transfer from a hydrophobic α-amino acid to a hydrophilic α-keto acid is more favourable than the reverse reaction in the cationic molecular aggregates.

Transamination of L-*Phe with Pyruvate.*—The transamination reaction of L-Phe with pyruvate proceeded with turnover behaviour of the catalyst system composed of copper(II) ions and the single-walled co-vesicle of $N^+C_5His2C_{16}$ and PL^+2C_{16}. As shown in Figure 1, an amount of L-Phe equimolar to PL^+2C_{16} was readily consumed in several hours and then the

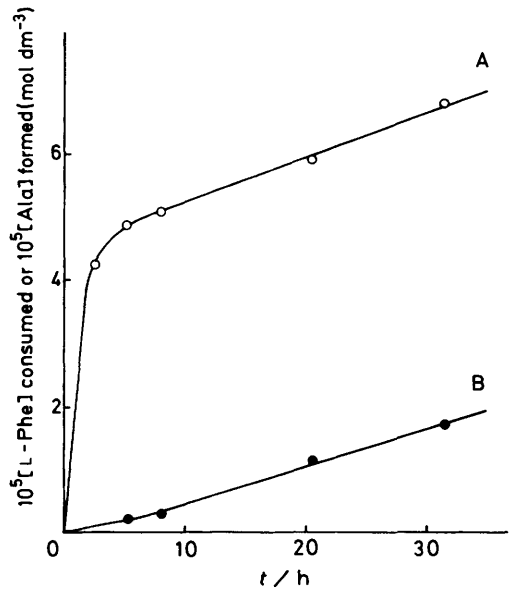


Figure 1. Time courses for transamination of L-Phe with pyruvate as catalysed by PL^+2C_{16}, copper(II) ions, and the $N^+C_5His2C_{16}$ vesicle in an aqueous acetate buffer (2.5×10^{-2} mol dm^{-3}, μ 0.10 with KCl) at pH 5.1 and 30.0 °C: A, consumption of L-Phe; B, formation of alanine. Initial concentrations in mol dm^{-3}: L-Phe, 4.7×10^{-4}; pyruvate, 4.7×10^{-3}; PL^+2C_{16}, 4.7×10^{-5}; $Cu(ClO_4)_2$, 4.7×10^{-5}; $N^+C_5His2C_{16}$, 1.0×10^{-3}

disappearance of L-Phe took place at much slower rate along with the formation of alanine; the extents of L-Phe consumption and Ala formation are nearly identical in the second step. The result means that the fast initial step in consumption of L-Phe corresponds to the transformation of PL^+2C_{16} and L-Phe into the corresponding pyridoxamine derivative (PM^+2C_{16}) and β-phenylpyruvic acid, respectively, and the catalytic cycle becomes operative in the slow second step.

Under the conditions shown in Figure 2, the yield of alanine in the $N^+C_5His2C_{16}$ vesicular system progressively increased in proportion to time. Even though the reaction rate was somewhat depressed after 48 h, the continuous production of alanine lasted at least over a month. The initial rates for formation of alanine are larger in the $N^+C_5Ala2C_{16}$ vesicle and the CTAB micelle than in a homogeneous system without amphiphiles. However, saturation behaviour was observed in these molecular aggregates within 48 h; maximum yields of alanine based on PL^+2C_{16} are 27 and 57% in the $N^+C_5Ala2C_{16}$ vesicle and the CTAB micelle, respectively (Figure 2). The imidazolyl group of $N^+C_5His2C_{16}$ definitely plays an important role in the transamination reaction. In the aqueous system involving PL^+C_1 (or PL) and copper(II) ions without amphiphiles, the transamination of L-Phe with pyruvate proceeded at a rate much slower than that in the $N^+C_5His2C_{16}$ vesicular system (Figures 2 and 3). An addition of imidazole to the homogeneous system scarcely affected the transamination rate (Figure 3).

The rate of alanine formation in the $N^+C_5His2C_{16}$ vesicular system increased with decrease in pH in the 5.1—9.3 range (Figure 4). On the other hand, the reaction rate increased with increase in pH in the aqueous system composed of PL^+C_1 and copper(II) ions: under the conditions comparable to those given in Figure 3, the yields of alanine after 24 and 72 h are 10 and 27%, respectively, at pH 5.1, and 16 and 49%, respectively, at pH 7.0. In the CTAB micellar system, the yield of alanine reached a maximum value (100%) after 24 h at pH 7.0 under conditions comparable to those shown in Figure 2.

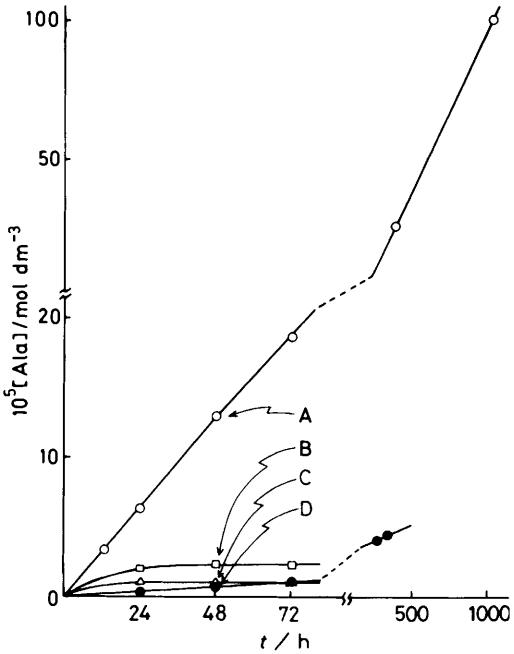


Figure 2. Time courses for transamination of L-Phe with pyruvate as catalysed by pyridoxal derivatives and copper(II) ions in an aqueous acetate buffer (2.5×10^{-2} mol dm^{-3}, μ 0.10 with KCl) at pH 5.1 and 30.0 °C: A, $PL^{+}2C_{16}$ in the $N^{+}C_5His2C_{16}$ vesicle; B, $PL^{+}2C_{16}$ in the CTAB micelle; C, $PL^{+}2C_{16}$ in the $N^{+}C_5Ala2C_{16}$ vesicle; D, $PL^{+}C_1$. Initial concentrations in mol dm^{-3}: L-Phe, 4.3×10^{-3}; pyruvate, 4.3×10^{-3}; pyridoxal derivatives, 4.3×10^{-5}; $Cu(ClO_4)_2$, 4.3×10^{-5}; peptide lipids, 1.0×10^{-3}; CTAB, 3.0×10^{-3}

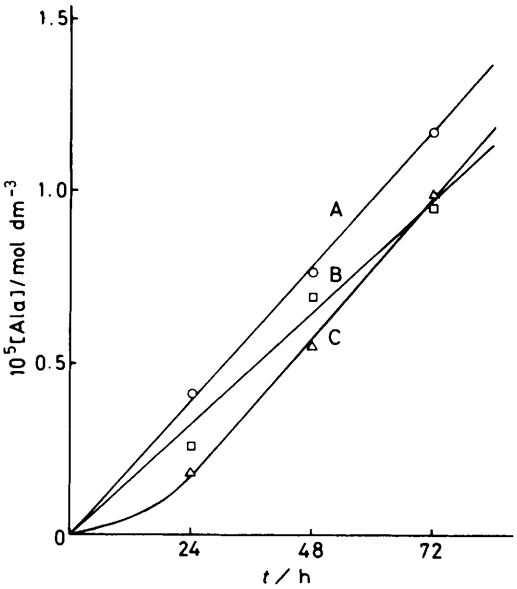


Figure 3. Time courses for transamination of L-Phe with pyruvate as catalysed by pyridoxal derivatives and copper(II) ions in an aqueous acetate buffer (2.5×10^{-2} mol dm^{-3}, μ 0.10 with KCl) at pH 5.1 and 30.0 °C: A, $PL^{+}C_1$; B, $PL^{+}C_1$ and imidazole (1.0×10^{-3} mol dm^{-3}); C, PL. Initial concentrations in mol dm^{-3}: L-Phe, 4.3×10^{-3}; pyruvate, 4.3×10^{-3}; pyridoxal derivatives, 4.3×10^{-5}; $Cu(ClO_4)_2$, 4.3×10^{-5}

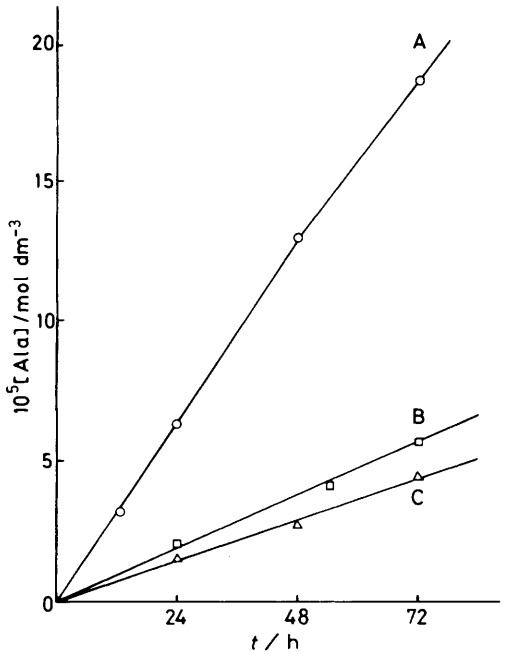


Figure 4. pH effect on transamination of L-Phe with pyruvate as catalysed by $PL^{+}2C_{16}$, copper(II) ions, and the $N^{+}C_5His2C_{16}$ vesicle at 30.0 °C in aqueous buffers (2.5×10^{-2} mol dm^{-3}, μ 0.10 with KCl): A, pH 5.1; B, pH 7.0; C, pH 9.3. Initial concentrations in mol dm^{-3}: L-Phe, 4.3×10^{-3}; pyruvate, 4.3×10^{-3}; $PL^{+}2C_{16}$, 4.3×10^{-5}; $Cu(ClO_4)_2$, 4.3×10^{-5}; $N^{+}C_5His2C_{16}$, 1.0×10^{-3}

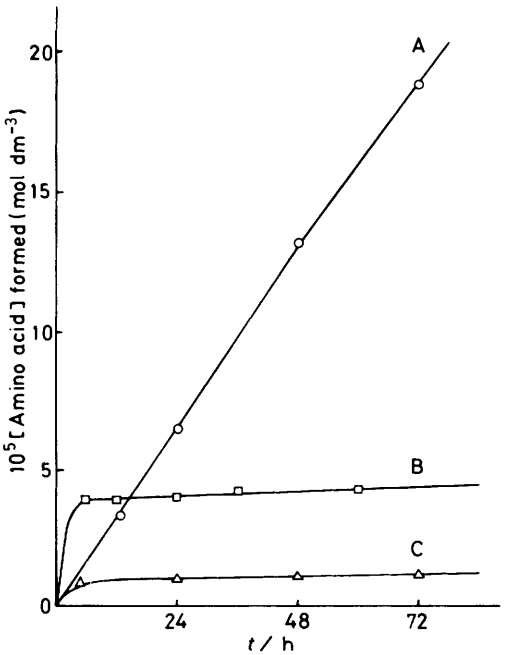


Figure 5. Time courses for transamination as catalysed by $PL^{+}2C_{16}$, copper(II) ions, and the $N^{+}C_5His2C_{16}$ vesicle in an aqueous acetate buffer (2.5×10^{-2} mol dm^{-3}, μ 0.10 with KCl) at pH 5.1 and 30.0 °C with the following combinations of α-amino acid and α-keto acid: A, L-Phe and pyruvate; B, L-Phe and α-ketoglutarate; C, L-Ala and β-phenylpyruvate. Initial concentrations in mol dm^{-3}: amino acids, 4.3×10^{-3}; keto acids, 4.3×10^{-3}; $PL^{+}2C_{16}$, 4.3×10^{-5}; $Cu(ClO_4)_2$, 4.3×10^{-5}; $N^{+}C_5His2C_{16}$, 1.0×10^{-3}

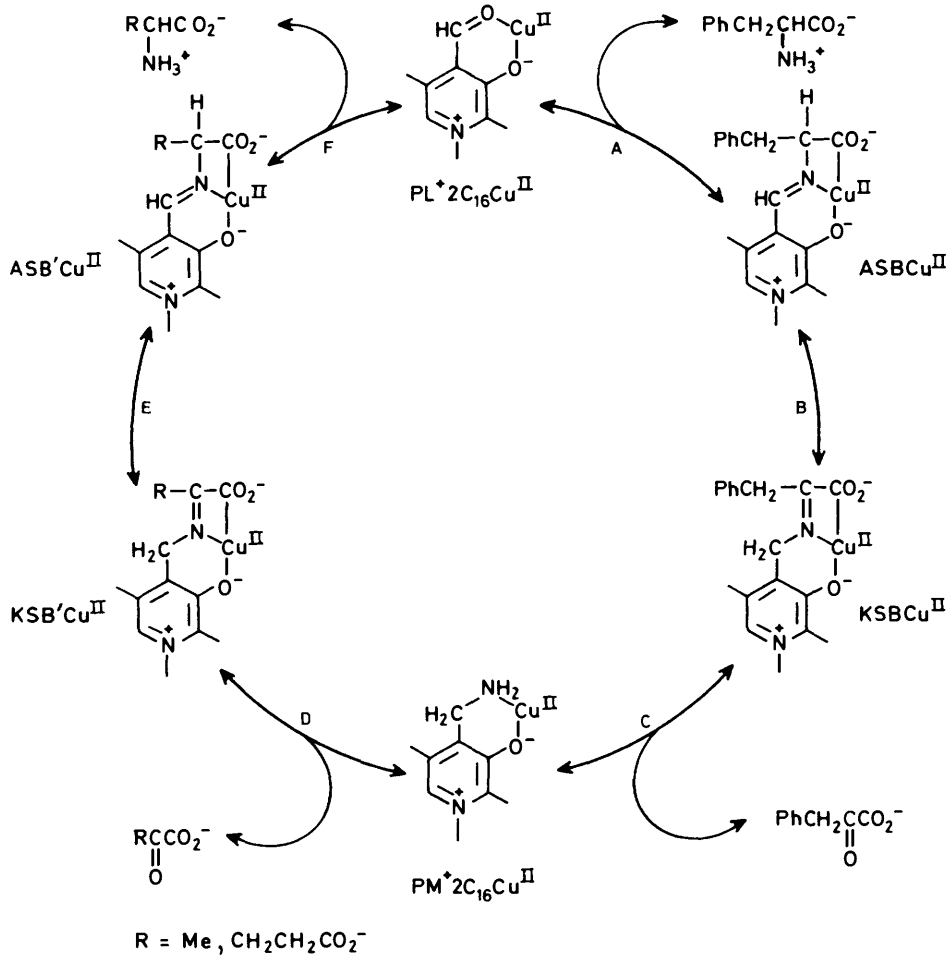


Figure 6. Schematic representation of transamination cycle with α-amino acids and α-keto acids as catalysed by $PL^{+}2C_{16}$ and copper(II) ions

For the reaction carried out in the $N^{+}C_5His2C_{16}$ vesicular phase, various combinations of an α-amino acid and an α-keto acid were employed to examine substrate selectivity at pH 5.1 and 30.0 °C. While the transamination of L-Phe with pyruvate effectively proceeded with turnover behaviour, the reverse reaction (L-Ala with β-phenylpyruvate) did not show such behaviour (Figure 5). As for the transamination of L-Phe with α-ketoglutarate, the initial rate of glutamic acid formation is somewhat larger than that of alanine formation for the combination of L-Phe and pyruvate. However, the formation of glutamic acid reached a saturation level after a certain period of time: 90 and 97% after 12 and 60 h, respectively (Figure 5). Thus, the present catalytic system is effective for the transamination reaction of a hydrophobic α-amino acid (L-Phe) with a hydrophilic α-keto acid (pyruvic acid).

Reaction Mechanism.—The validity of the reaction scheme shown in Figure 6 is approved on the basis of the following experimental findings. The transamination of $PL^{+}2C_{16}$ with L-Phe in the single-walled vesicle of $N^{+}C_5His2C_{16}$ is markedly accelerated by the catalytic assistance of copper(II) ions at pH 10 and 30.0 °C[11] (steps A ⟶ B ⟶ C in Figure 6). These sequential reactions also proceed similarly at pH 5.1, judging from the reaction behaviour shown in Figure 1. We have also studied the copper(II)-catalysed transamination of hydrophobic pyridoxamines, PMC_{12} and $PM2C_{16}$, with pyruvate in vesicles at pH 7[9,12,13] (corresponding steps D ⟶ E ⟶ F in Figure 6). The reaction readily proceeds to afford the copper(II) chelate of the aldimine Schiff's base. However, the subsequent hydrolysis of the Schiff's base does not occur unless ethylenediaminetetra-acetate is added. Similar reaction behaviour was observed spectrophotometrically at pH 5 for the $PM2C_{16}$–$N^{+}C_5Ala2C_{16}$ system involving copper(II) ions; the aldimine Schiff's base chelate is protonated at the pyridyl nitrogen (pK_a 6.5) and an electron-withdrawing effect originated from the protonated pyridyl nitrogen may be expected. In the light of the above observations, the hydrolysis of the aldimine Schiff's base chelate to afford an amino acid and $PL^{+}2C_{16}$ (step F in Figure 6) seems to become the rate-determining step in the present overall transamination reaction.

This conclusion is supported by the fact that the turnover behaviour can not be observed for the reaction in the $N^{+}C_5Ala2C_{16}$ vesicle and the CTAB micelle (Figure 2). We confirmed spectrophotometrically that the aldimine chelate ($ASB'Cu^{II}$) with an absorption maximum at 380 nm is present in these reaction mixtures and its amount equals to the yield of alanine evaluated after dansylation of the products; an amount of the dansylated amino acid corresponds exactly to that of the $PL^{+}2C_{16}$-bound alanine. On the other hand, the aldimine Schiff's base chelate in the $N^{+}C_5His2C_{16}$ vesicle is effectively hydrolysed by the catalytic assistance of the imidazolyl group placed in the close vicinity of the aldimine chelate to generate $PL^{+}2C_{16}$ and alanine. Under the reaction conditions shown in Figure 2, the amount of the aldimine Schiff's base chelate in the

$N^+C_5His2C_{16}$ vesicle is much smaller than those in the $N^+C_5Ala2C_{16}$ vesicle and the CTAB micelle, as observed by electronic absorption spectroscopy. However, step F in Figure 6 still remains as the rate-determining step in the $N^+C_5His2C_{16}$ vesicle, since the rate of alanine formation becomes faster in the lower pH region (Figure 4) where the hydrolysis of the aldimine Schiff's base chelate is much favoured.

As for the transamination of L-Phe with pyruvate in the $N^+C_5His2C_{16}$ vesicular system, the catalytic cycle shown in Figure 6 must rotate clockwise. In the first place, the copper(II) chelate of PL^+2C_{16} ($PL^+2C_{16}Cu^{II}$) reacts with L-Phe to afford the copper(II) chelate of PM^+2C_{16} ($PM^+2C_{16}Cu^{II}$) and β-phenylpyruvic acid (steps A ⟶ B ⟶ C). An extent of formation of the ketimine Schiff's base chelate of PM^+2C_{16} with an α-keto acid, pyruvate or β-phenylpyruvate (step D or C), depends on the stability constant for the complex ($KSB'Cu^{II}$ or $KSBCu^{II}$) and the concentration of each α-keto acid. The stability constant for $KSBCu^{II}$ is considered to be larger than that for $KSB'Cu^{II}$ in the quasi-hydrophobic reaction site provided in the vesicle* since β-phenylpyruvate is more hydrophobic than pyruvate. However, pyruvate, which has a hydrophilic nature, is concentrated in the cationic vesicular surface due to the electrostatic interaction to give a relatively large fraction of the ketimine Schiff's base even in the absence of copper(II) ions. At an early stage of the transamination reaction when the concentration of β-phenylpyruvate is much lower than that of pyruvate, step D may proceed more favourably than C. The facile hydrolysis of the aldimine Schiff's base chelate ($ASB'Cu^{II}$) to afford $PL^+2C_{16}Cu^{II}$ and alanine (step F) is the key process for execution of turnover behaviour. Since the stability constant for the aldimine Schiff's base of PL^+2C_{16} with L-Ala is much smaller than that for the Schiff's base of PL^+2C_{16} with L-Phe,[11] $ASB'Cu^{II}$ is more readily hydrolysed to $PL^+2C_{16}Cu^{II}$ and alanine than the aldimine Schiff's base chelate of PL^+2C_{16} with phenylalanine ($ASBCu^{II}$) is done. In the same sense, $PL^+2C_{16}Cu^{II}$ thus regenerated preferentially forms $ASBCu^{II}$ rather than $ASB'Cu^{II}$. As a result, the catalytic cycle shown in Figure 6 effectively rotates clockwise (Figure 5).

The transamination of L-Ala with β-phenylpyruvate, which requires the counter-clockwise sequence in Figure 6, is extremely less favourable in the light of the above discussion. As a result, $ASBCu^{II}$ is produced only in a limited yield (23% after 24 h) in the sequential reactions from step F to B, and the extremely slow hydrolysis of $ASBCu^{II}$ is followed (Figure 5).

As for the amino-group transfer from L-Phe to α-ketoglutaric acid, the formation of $KSB'Cu^{II}$ from $PM^+2C_{16}Cu^{II}$ and α-ketoglutarate (step D) takes precedence over the formation of $KSB'Cu^{II}$ from $PM^+2C_{16}Cu^{II}$ and pyruvate due to the much more favourable electrostatic interaction of the dianionic α-ketoglutarate with the cationic vesicular surface. In fact, the initial rate of $ASB'Cu^{II}$ formation in the former system, evaluated on the basis of the amount of glutamic acid after dansylation of the reaction mixture, is larger than that in the latter; the yield of $ASB'Cu^{II}$ reaches as much as 90% after 12 h (Figure 5). However, the hydrolysis of $ASB'Cu^{II}$ to afford $PL^+2C_{16}Cu^{II}$ and glutamic acid (step F) is much depressed, and the turnover behaviour was not observed within the time range investigated here.

In conclusion, the transamination of a hydrophobic α-amino acid (L-Phe) with a hydrophilic α-keto acid (pyruvate) was catalysed by the artificial transaminase composed of PL^+2C_{16}, $N^+C_5His2C_{16}$, and copper(II) ions under mild conditions, showing turnover behaviour. The catalytic efficiency of this artificial enzyme is primarily governed by the product-releasing process, the hydrolysis of the copper(II) chelate of the aldimine Schiff's base to afford an α-amino acid as the product. Addition of the metal ions is essential to enhancement of the isomerization reactivity in the non-enzymic systems. However, the coordination of the copper(II) ion results in inactivation of the aldimine Schiff's base (ASB') toward its hydrolysis. In order to overcome this disadvantage and develop more active artificial transaminases, replacement of metal ions with some protic components, which may accelerate both isomerization and product-releasing processes, is required in the manner observed in the enzymic systems.

* The pyridoxal moiety is placed in a microenvironment equivalent to that provided by propan-1-ol.[11]

Experimental

Elemental analyses were performed at the Microanalysis Centre of Kyushu University. A Beckman Φ71 pH meter equipped with a Beckman 39505 combined electrode was used for pH measurements after calibration with a combination of appropriate standard aqueous buffers. Electronic absorption spectra were obtained with a Union Giken SM-401 high-sensitivity spectrophotometer.

Materials.—The following α-amino acids and α-keto acids were obtained from commercial sources as guaranteed reagents and used without further purification: L-phenylalanine (Protein Research Foundation, Osaka, Japan), L-alanine (Nakarai Chemicals, Kyoto, Japan), L-glutamic acid (Wako Pure Chemical Industries, Osaka, Japan), sodium β-phenylpyruvate (Sigma Chemical Co., Missouri, U.S.A.), sodium pyruvate, and α-ketoglutarate (both from Wako Pure Chemical Industries). Pyridoxal hydrochloride (PL) was obtained from Mann Research Laboratories, New York, U.S.A. 1,2-Dimethyl-3-hydroxy-4-formyl-5-hydroxymethylpyridinium chloride (PL^+-C_1) was prepared after the method of Heyl *et al.*[15] [Found: C, 48.55; H, 5.55; N, 6.25. Calc. for $C_9H_{12}ClNO_3\cdot(\frac{1}{5})H_2O$: C, 48.55; H, 5.65; N, 6.35%]. Preparation and characterization of 1-(*NN*-dihexadecylcarbamoylmethyl)-2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridinium chloride (PL^+2C_{16}),[16] *NN*-dihexadecyl-N^a-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5His2C_{16}$),[9] *NN*-dihexadecyl-N^a-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+-C_5Ala2C_{16}$),[17] and *NN*-ditetradecyl-N^a-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala-2C_{14}$)[17] have been reported elsewhere. Hexadecyltrimethylammonium bromide (CTAB) (Nakarai Chemicals) was recrystallized from ethanol, m.p. 237—239 °C (decomp.). Imidazole (Nakarai Chemicals) was purified by sublimation at 60 °C at 0.01 mmHg. Dansyl chloride for fluorescent labelling of amino acids was obtained from Nakarai Chemicals as a guaranteed reagent. Copper(II) perchlorate (Kishida Chemical Co., Osaka, Japan) was dissolved in deionized water and standardized by conventional chelatometric titration.

Preparation of Single-walled Co-vesicles.—An appropriate amount of stock solutions of the amphiphiles ($N^+C_5His2C_{16}$ and $N^+C_5Ala2C_{16}$) in ethanol was placed in a glass vessel, and the solvent was removed completely *in vacuo*. An appropriate amount of a chloroform solution of PL^+2C_{16} was added to the residue and evaporated to dryness *in vacuo* at room temperature. The residue placed in an appropriate amount of aqueous buffers (2.5×10^{-2} mol dm^{-3}, μ 0.10 with KCl) afforded an aqueous dispersion by vortex mixing with glass beads for 1 min at room temperature. The following aqueous buffers were used in the present study: pH 5.1, acetate; pH 7.0, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulphonate (HEPES); pH 9.3, borate. A clear solution involving single-walled vesicles was prepared upon sonication of the dispersion for 1 min with a probe-type sonicator (W-220F, Heat Systems-Ultrasonics) at

30 W and room temperature. All the vesicle solutions did not undergo any turbidity change for more than several weeks. The detailed procedure for morphological characterization of the single-walled vesicles will be reported elsewhere.[16]

Kinetic Measurements.—Each run was initiated by adding aqueous solutions of an α-amino acid (200 μl), an α-keto acid (100 μl), and $Cu(ClO_4)_2$ (10 μl) to the co-vesicle solution (2.0 ml) which was pre-equilibrated at 30.0 ± 0.1 °C in a thermostatted cell. The initial concentrations of chemical species were set as follows for most of the runs: α-amino acids, 4.3×10^{-3}; α-keto acids, 4.3×10^{-3}; PL^+2C_{16}, 4.3×10^{-5}; $Cu(ClO_4)_2$, 4.3×10^{-5}; peptide lipids, 1.0×10^{-3} mol dm^{-3}. The reaction was followed by measuring the amounts of amino acids either unchanged or produced in the course of the transamination. The amino acids, which were present as free species and/or as the aldimine Schiff's base chelates, were dansylated and quantitatively analysed by reversed-phase h.p.l.c.[18] as follows. An aliquot sample (50 μl) was taken from the mixture at an appropriate time, and mixed with an aqueous borate buffer (250 μl, 5.0×10^{-2} mol dm^{-3}, pH 9.3). An acetonitrile solution of dansyl chloride (150 μl, 5.0×10^{-3} mol dm^{-3}) was then immediately added to the mixture, which was subsequently allowed to stand for 1 h in the dark at room temperature. The dansylated amino acids were separated on a column of TSK gel ODS-120T (length, 250 mm; internal diameter, 4.6 mm) with a Hitachi 635 liquid chromatograph, and the eluting fractions were monitored by a Hitachi F-1000 fluorescence spectrophotometer: flow rate, 0.5—0.6 ml min^{-1}; mobile phase, mixtures of Tris buffer (1.0×10^{-2} mol dm^{-3}, pH 8.1) and methanol at volume ratios of 10:3, 2:1, and 1:2 for dansylated glutamic acid, alanine, and phenylalanine, respectively. The calibration curves for quantitative analysis were established by using a series of sample solutions containing known amounts of amino acids and other components involved in respective kinetic runs except PL^+2C_{16}, which were subjected to the identical treatments mentioned above. In order to confirm the analytical reliability, ethylenediaminetetra-acetate was added to the mixture before dansylation for selected cases. Since the identical results were obtained without such a chelating agent, the copper(II) ion does not interfere with the dansylation reaction. Analytical reproducibility was confirmed to be satisfactory.

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