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Stereoselective Synthesis of β-Phenylserine from Glycine and Benzaldehyde as Mediated by Catalytic Bilayer Membranes, Artificial Vitamin B_6-dependent Enzymes

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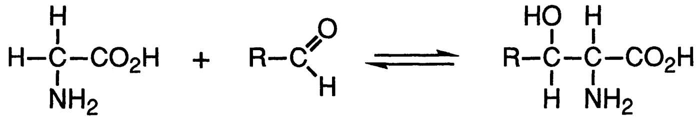
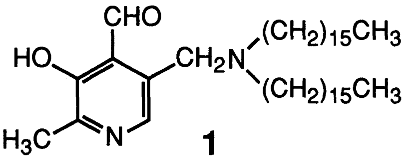
The aldolation of glycine with benzaldehyde to yield β-phenylserine was catalyzed by supramolecular assemblies formed with a synthetic peptide lipid, a hydrophobic vitamin B_6 derivative, and zinc(II) ions in aqueous media under mild conditions. A diastereomeric ratio of the reaction products was much dependent on a size and a phase property of the vesicular catalyst.

The development of artificial enzymes is one of the most important research targets in the field of biomimetic chemistry.1-3) We have recently shown that bilayer membranes formed with synthetic peptide lipids provide appropriate reaction sites for mimicking various enzymatic reactions.4) When a hydrophobic coenzyme analogue having vitamin B_6 activity is anchored noncovalently in the interior membrane domain, the resulting artificial enzymes are capable of simulating catalytic functions as observed with vitamin B_6-dependent transaminase and tryptophan synthase.4-6) In this communication, we are to report that bilayer membranes having vitamin B_6 activity are also effective in promoting another reaction, aldolation of glycine (Gly) with an aldehyde to form the corresponding β-substituted serine derivative (refer to Eq. 1).

Serine hydroxymethyltransferase is an aldolase requiring pyridoxal 5'-phosphate (PLP) as a coenzyme, and catalyzes interconversions between Gly and various β-hydroxy-α-amino acids, such as serine, threonine, and β-phenylserine (β-PhSer), via formation of a carboanion intermediate derived from the enzyme-bound Schiff-base of PLP with the amino acid substrate.7,8) Although the aldolation of Gly with aldehydes to form the corresponding β-hydroxy-α-amino acids occurs nonenzymatically in aqueous alkaline media in the presence and absence of vitamin B_6 analogues and/or metal ions,9-11) any efficient artificial catalyst for the


$$\mathrm{H{-}\underset{NH_2}{\overset{H}{C}}{-}CO_2H} + \mathrm{R{-}C{\overset{O}{\underset{H}{\lessgtr}}}} \rightleftharpoons \mathrm{R{-}\underset{H}{\overset{HO}{C}}{-}\underset{NH_2}{\overset{H}{C}}{-}CO_2H} \qquad (1)$$



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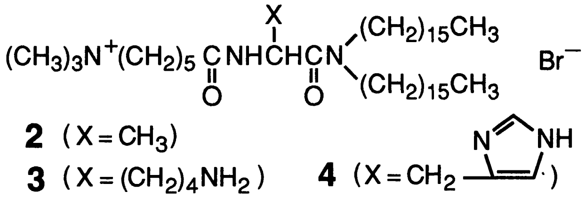

$(CH_3)_3N^+(CH_2)_5\underset{\|}{C}NH\overset{X}{C}H\underset{\|}{C}N\langle^{(CH_2)_{15}CH_3}_{(CH_2)_{15}CH_3}\ Br^-$

2 ($X = CH_3$)

3 ($X = (CH_2)_4NH_2$)

4 ($X = CH_2$–imidazolyl)

aldolation in neutral pH media under mild conditions has not been reported up to the present time.

We now evaluated the catalytic efficiency of functionalized bilayer membranes formed with a hydrophobic pyridoxal derivative (**1**)[6] and a synthetic peptide lipid (**2**,[12] **3**,[13] or **4**[14]) for the aldolation reaction of Gly with benzaldehyde in an aqueous medium at pH 7.0 and 30.0 °C in the presence and absence of metal ions. Progress of the reaction was monitored by measuring amounts of β-PhSer produced, which was dansylated[5] and separated by HPLC on a column of TSK gel ODS-120T with methanol–aqueous HEPES buffer (10 mmol dm^{-3}, pH 7.5) at a 1:1 v/v ratio as eluant. The two diastereomers of β-PhSer, *erythro*- and *threo*-forms [a mixtures of (2*R*,3*R*)- and (2*S*,3*S*)- and a mixture of (2*R*,3*S*)- and (2*S*,3*R*)-forms, respectively], were well resolved under the present HPLC conditions. We found that the aldolation to form β-PhSer proceeded by cooperative catalysis of a pyridoxal derivative and zinc(II) ions even under the mild conditions employed here.

While an extent of the aldolation catalyzed by PLP and zinc(II) ions in homogeneous aqueous solution is extremely low (Fig. 1, line A), the bilayer vesicle composed of **1**, **2**, and zinc(II) ions with a hydrodynamic diameter (d_{hy}) of 120 nm drastically accelerated the reaction, showing turnover behavior (Fig. 1, line B). Such enhancement by the bilayer catalyst is presumably attributed to the entropy gain generated by the supramolecular effect: an efficient attack of the hydrophobic benzaldehyde molecule on the carbanion intermediate (**6**), which is derived by deprotonation of the aldimine Schiff-base chelate (**5**) embedded in the membrane (Scheme 1).

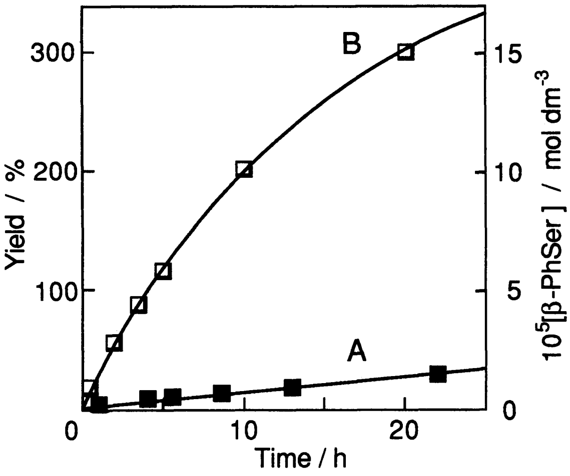
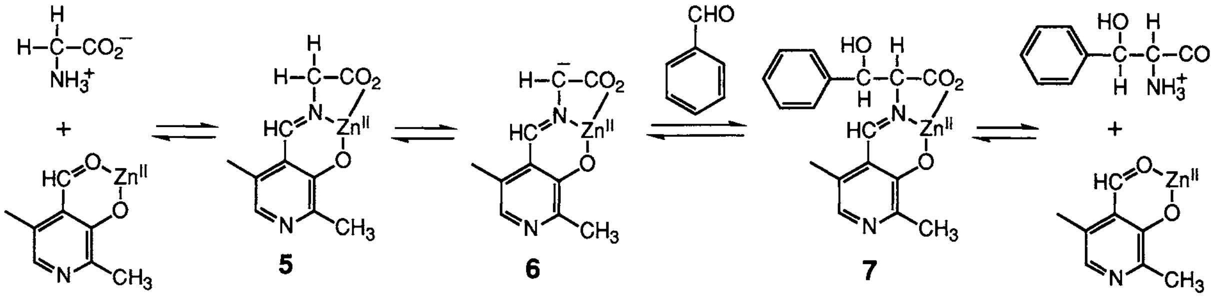


Fig. 1. Time courses for aldolation of Gly (5.0 mmol dm^{-3}) with benzaldehyde (15.0 mmol dm^{-3}) to form β-PhSer as catalyzed by pyridoxal derivatives and zinc(II) ions (0.050 mmol dm^{-3} each) in aqueous media at pH 7.0 and 30.0 °C: A, PLP; B, **1** embedded in the bilayer aggregate of **2** (1.0 mmol dm^{-3}) with a d_{hy} value of 120 nm.

Reactivity values, which were evaluated on the basis of initial reaction rates for formation of the total β-PhSer, and diastereomeric excess (d.e.) values of *threo*-β-PhSer relative to the corresponding *erythro*-form were measured under various conditions and the results are listed in Table 1. The catalytic activity of the bilayer assembly was



Scheme 1.

lowered by replacement of peptide lipid component **2** by **3** or **4** (Table 1, Entries 3, 7, and 8). We evaluated the p*K* values for ε-amino group of the lysine residue of **3** and imidazolyl group of the histidine residue of **4** in the bilayer membrane at 30.0 °C by means of ^{1}H-NMR titration to give 9.3 and 4.5, respectively. Thus, the ε-amino group of **3** and the imidazolyl group of **4** are capable of acting as general acid and base catalysts, respectively, under the present reaction conditions at pH 7.0. Nevertheless, the observed lower reactivity values for the aldolation imply that the reaction accompanies another type of side reactions, e.g. transamination, which effectively receives an assistance of general acid-base catalysis.[5,15)]

The diastereoselectivity, as exposed by the bilayer catalyst formed with **1**, **2**, and zinc(II) ions with a d_{hy} value of 120 nm, is somewhat higher than that catalyzed by PLP and zinc(II) ions

Table 1. Reactivity and diastereoselectivity values for aldolation of Gly with benzaldehyde to form β-PhSer in aqueous media at pH 7.0 [a)]

Entry	Catalyst	Temp/°C	d_{hy}/nm[b)]	Reactivity[c)]	d.e./%[d)]
1	PLP - Zn^{II}	30.0	—[e)]	100[h)]	22
2		10.0	—[e)]	7	27
3	**1** - **2** - Zn^{II}	30.0	120[f)]	1640	30
4		30.0	260[g)]	220	42
5		10.0	110[f)]	140	56
6		10.0	260[g)]	15	54
7	**1** - **3** - Zn^{II}	30.0	130[f)]	1310	34
8	**1** - **4** - Zn^{II}	30.0	110[f)]	760	38

a) Initial concentrations in mmol dm^{-3}: Gly, 5.0; benzaldehyde, 15.0; pyridoxal derivatives, 0.050; peptide lipids, 1.0; Zn(NO$_3$)$_2$, 0.050. b) Evaluated by means of dynamic light scattering measurements. c) Analyzed by HPLC and evaluated on the basis of initial reaction rate for formation of the total β-PhSer. d) Diastereomeric excess (d.e.) of *threo*-β-PhSer relative to the corresponding *erythro*-form as determined by HPLC. e) No aggregate formation. f) Sonicated with a probe-type sonicator for 30 s at 30 W. g) Aqueous dispersion state. h) Initial rate, 6.7×10^{-7} mol dm^{-3} h^{-1}.

in homogeneous aqueous solution at 30.0 °C (Table 1, Entries 1 and 3). The d.e. value was much increased by using the catalytic vesicles with a larger size in the dispersion state at 30.0 °C, although the reactivity was largely reduced in the internal domain of the multiwalled vesicle (Table 1, Entry 4). The phase transition from gel to liquid-crystalline state occurs in a range of 15–25 °C for the bilayer membrane formed with **1** and **2**.$^{14)}$ Thus, the catalyst activity was examined in the liquid-crystalline and the gel state at 30.0 and 10.0 °C, respectively. The relatively high d.e. values were observed in the gel state regardless of the vesicular size (Table 1, Entries 5 and 6). Accordingly, the diastereoselectivity in various reaction media increased in the following order in reflection of molecular organization of the reaction site matrix: homogeneous aqueous media, a small bilayer vesicle in the liquid-crystalline state, a large bilayer vesicle in the liquid-crystalline state, and a bilayer vesicle in the gel state regardless of its size.

In conclusion, it became apparent that the catalytic bilayer membrane formed with **1**, **2**, and zinc(II) ions can be utilized as a supramolecular assembly for simulation of an enzymatic reaction catalyzed by the vitamin B_6-dependent aldolase.

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