Catalytic Performance of a Supramolecular Bienzyme Complex Formed with Artificial Aminotransferase and Natural Lactate Dehydrogenase

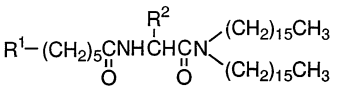
Jun-ichi Kikuchi,* Yasuyuki Kamijyo, Hiroshi Etoh, and Yukito Murakami*
Institute for Fundamental Research in Organic Chemistry, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-81

(Received February 19, 1996)

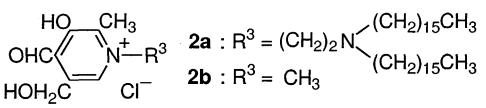
A supramolecular bienzyme complex was constituted in a combination of a catalytic bilayer membrane as an artificial aminotransferase with a natural lactate dehydrogenase and the resulting cooperative system enhanced the catalytic activity of the artificial enzyme to promote a sequential transformation of α-amino acids to the corresponding α-hydroxy acids via formation of α-keto acids, exhibiting a marked substrate specificity.

In connection with catalytic functions of enzymes, we have developed artificial holoenzymes by anchoring coenzyme factors noncovalently in synthetic bilayer membranes that mimic apoenzyme functions.[1] In this regard, we have recently demonstrated that catalytic functions performed by naturally occurring vitamin B_6-dependent enzymes can be simulated by artificial supramolecules constituted with a bilayer-forming peptide lipid, a hydrophobic vitamin B_6 derivative, and metal ions. Especially, a functionalized bilayer vesicle formed with a peptide lipid having an L-lysine residue (**1a**) and a hydrophobic pyridoxal derivative (**2a**) was found to act as an efficient artificial aminotransferase (AT) showing both turnover behavior and high enantioselectivity in the presence of copper(II) ions.[2] We are now focusing our work on manipulation of multienzyme systems in combinations of the artificial enzyme with natural ones. We report here that a supramolecular bienzyme system constituted in a combination of the artificial AT with a natural lactate dehydrogenase (LDH) enhances a sequential transformation of α-amino acids to the corresponding α-hydroxy acids via formation of α-keto acids as shown by Eq. 1.

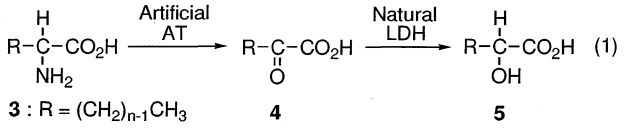
We have previously shown that the half-transamination reaction of α-amino acids to afford the corresponding α-keto acids was catalyzed by functionalized bilayer vesicles formed with a peptide lipid and a hydrophobic pyridoxal derivative quaternized at the pyridyl nitrogen in aqueous media in the presence of various metal ions.[3] The reaction proceeded through formation of a metal complex of an aldimine Schiff-base derived from a hydrophobic pyridoxal and an α-amino acid, isomerization of the aldimine metal chelate to the corresponding ketimine chelate, and hydrolysis of the ketimine chelate to afford a hydrophobic pyridoxamine and an α-keto acid, in this sequence. We



1a : $R^1 = (CH_3)_3N^+$ Br^-, $R^2 = (CH_2)_4NH_2$
1b : $R^1 = (CH_3)_3N^+$ Br^-, $R^2 = CH_3$
1c : $R^1 = Na^+$ (SO_3^-)—, $R^2 = CH_3$



2a : $R^3 = (CH_2)_2N$(…)
2b : $R^3 = CH_3$



3 : $R = (CH_2)_{n-1}CH_3$ **4** **5** (1)

found that the rate-determining step in the half-transamination was the isomerization that is unfavorable not only thermodynamically but also kinetically. In order to overcome such unfavorable conditions and to enhance the half-transamination reaction, we designed a novel reaction system by coupling the reaction with a product-consuming reaction, such as reduction of an α-keto acid to afford the corresponding α-hydroxy acid. In this regard, we now examined the catalytic efficiency of a bienzyme system composed of the artificial AT and an NADH-dependent oxidoreductase, pig heart LDH (Boehringer Manheim, Germany).

A hydrodynamic diameter (d_{hy}) evaluated by means of dynamic light scattering measurements for single-walled bilayer vesicles of **1a** (1.0 mmol dm^{-3}), as prepared by sonication of the aqueous dispersion with a probe-type sonicator at 30 W for 30 s, was 60 nm in an aqueous HEPES buffer (25 mmol dm^{-3}, μ 0.1 with KCl) at pH 7.0 and 30.0 °C. Upon addition of LDH (8.3 mg dm^{-3}) to the vesicular solution, the d_{hy} value increased to 150 nm, reflecting formation of a complex of the vesicle with LDH. The diameter underwent a change from 60 to 240 nm in the KCl-free solution upon addition of the same amount of LDH. While the similar behavior was observed with cationic vesicles of **1b** having an L-alanine residue in place of **1a**, the d_{hy} value for anionic vesicles of **1c** remained constant without any effect by the presence of LDH. Accordingly, the cationic vesicles of these peptide lipids as artificial apoenzymes are capable of forming multifunctional aggregates with LDH mainly through electrostatic interactions. In addition, the phase transition behavior from gel to liquid-crystalline state for the single-walled vesicles was not influenced upon addition of LDH, as evaluated by means of differential scanning calorimetry (DSC). On the basis of such observations, we can eliminate any possibility of LDH-induced fusion of the present single-walled vesicles to give multiwalled bilayer membranes.[4]

An enzymic activity of LDH in the reduction of α-keto acids is usually evaluated spectrophotometrically at 340 nm by following a consumption rate of NADH.[5] In the present bienzyme system, however, this assay method was not adopted because the absorption spectral changes for the transamination and the reduction were overlapped each other. Under such conditions, we employed HPLC to follow the sequential reactions by direct measurements of both NADH and its oxidized form (NAD) at 260 nm on a column of TSKgel Super-ODS (TOSOH corporation) with acetonitril–phosphate buffer (50 mmol dm^{-3}, pH 7.0) at a 1:100 v/v ratio as eluant.

In reference to the above observations, the present bienzyme system was constituted with the artificial AT and the natural LDH by employing the following components: **1a** (1.0 mmol dm^{-3}), **2a** (0.05 mmol dm^{-3}), and $Zn(ClO_4)_2$ (0.05 mmol dm^{-3}) for the artificial AT; LDH (8.3 mg dm^{-3}) and NADH (0.05 mmol dm^{-3}) for the LDH holoenzyme.[6] Time courses for the transformation of DL-norleucine [**3**(n=4)] as mediated by the bienzyme complex and the artificial AT alone are shown in Figure 1.[7] Since the reaction rate for reduction by LDH is much faster than that for transamination by the artificial AT under the present conditions, the plot in Figure 1A reflects the catalytic activity of the artificial AT involved in the bienzyme complex. As for the bienzyme system, the extents of both NADH consumption and NAD formation showed a good agreement with each other within 3% accuracy for each run. In the case of the LDH-free system (Figure 1B), the yield of **4**(n=4) was evaluated spectrophotometrically by the enzyme assay using LDH under the conditions that the α-keto acid is reduced by NADH immediately. The result clearly indicates that the bienzyme system enhances the catalytic activity of the artificial enzyme to promote the sequential transformation of α-amino acids to the corresponding α-hydroxy acids via formation of α-keto acids. In addition, the sequential reactions did not proceed at all in a homogeneous aqueous system in which **2a** was replaced by **2b** without **1a**.

The catalytic activity of the bienzyme system was much reduced when **1b** replaced **1a** as the peptide lipid (Table 1, entries 1 and 2), reflecting a fact that the ε-amino group of the lysine residue is responsible for significant catalytic assistance. Since the mediator reactivity of the bienzyme system containing the cationic **1b** was greater than that of the system containing the anionic **1c**, some catalytic cooperation is taking place between the artificial AT and the natural LDH through formation of the bienzyme complex in the former system (Table 1, entries 2 and 3). It is noteworthy that an effective bienzyme complex can not be constituted with LDH and the cationic hexadecyltrimethylammonium bromide (CTAB) micelle due to a fact that an interaction between the surfactant molecules and LDH causes denaturation of the protein, resulting in loss of the catalytic activity.

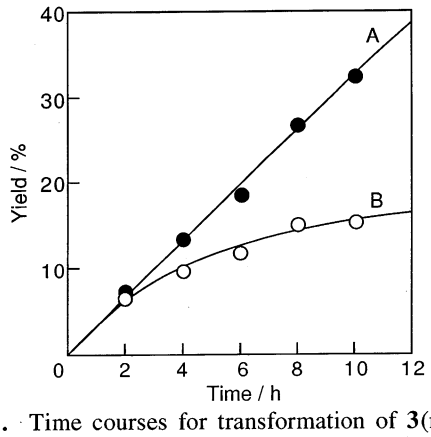


Figure 1. Time courses for transformation of **3**(n=4) (5.0 mmol dm^{-3}) to: (A), **5**(n=4) as catalyzed by a bienzyme complex of AT with LDH; (B), **4**(n=4) as catalyzed by AT in the absence of LDH; in an aqueous HEPES buffer (25 mmol dm^{-3}, μ 0.1 with KCl) at pH 7.0 and 30.0 °C. AT was constituted with **1a**, **2a**, and Zn(II) ions (1.0, 0.050, and 0.050 mmol dm^{-3}, respectively), while LDH was a holoenzyme formed with the apoenzyme (8.3 mg dm^{-3}) and NADH (0.050 mmol dm^{-3}). Yields are based on the amount of **2a**.

Table 1. Catalytic activities of bienzyme complexes formed with artificial AT and natural LDH for transformation of α-amino acids to the corresponding α-hydroxy acids at 30.0 °C[a]

Entry	Substrate	Peptide lipid	pH[b]	Relative reactivity[c]
1	**3**(n=4)	**1a**	7.0	100[d]
2	**3**(n=4)	**1b**	7.0	22
3	**3**(n=4)	**1c**	7.0	17
4	**3**(n=3)	**1a**	7.0	53
5	**3**(n=2)	**1a**	7.0	40
6	**3**(n=1)	**1a**	7.0	46
7	**3**(n=4)	**1a**	7.9	190
8	**3**(n=4)	**1a**	8.3	220
9	**3**(n=4)	**1a**	8.8	295
10	**3**(n=4)	**1a**	9.3	140

[a]Concentrations: α-amino acid, **1**, **2a**, $Zn(ClO_4)_2$, and NADH (5.0, 1.0, 0.050, 0.050, and 0.050 mmol dm^{-3}, respectively); LDH (8.3 mg dm^{-3}). [b]In aqueous HEPES and CHES buffers (25 mmol dm^{-3}, μ 0.1 with KCl) at pH 7.0–7.9 and 8.3–9.3, respectively. [c]Analyzed by HPLC for NAD and NADH and evaluated on the basis of initial reaction rates. [d]Initial rate, 1.8×10^{-6} mol dm^{-3} h^{-1}.

The bienzyme complex containing **1a** exhibited marked substrate specificity (Table 1, entries 1, 4–6). The selectivity seems to be controlled by the artificial AT, since the LDH activity decreases in the order: **4**(n=1) > **4**(n=2) > **4**(n=3) > **4**(n=4).[7] We also observed that the catalytic activity of the bienzyme complex showed marked pH dependence (Table 1, entries 1, 7–10).

In conclusion, we demonstrated here the first example of supramolecular multienzyme systems constituted in combinations of an artificial enzyme with a natural one. The detailed characterization of supramolecular effects is now in progress in our laboratory.

References and Notes

1 Y. Murakami and J. Kikuchi, in "Bioorganic Chemistry Frontiers," ed by H. Dugas, Springer-Verlag, Berlin (1991), Vol. 2, p. 73; Y. Murakami, J. Kikuchi, Y. Hisaeda, and O. Hayashida, *Chem. Rev.*, in press.
2 J. Kikuchi, Z.-Y. Zhang, and Y. Murakami, *J. Am. Chem. Soc.*, **117**, 5383 (1995).
3 Y. Murakami, J. Kikuchi, K. Akiyoshi, and T. Imori, *J. Chem. Soc., Perkin Trans. 2*, **1986**, 1445.
4 On fusion behavior of the single-walled vesicles formed with the peptide lipids as evaluated by DSC, see: Y. Murakami, J. Kikuchi, and T. Takaki, *Bull. Chem. Soc. Jpn.*, **59**, 3145 (1986).
5 J. J. Holbrook, A. Liljas, S. J. Steinsel, and M. G. Rossmann, in "The Enzymes," ed by P. D. Boyer, Academic Press, New York (1975), 3rd ed, Vol. 11, p. 191.
6 Although copper(II) ions are more effective than zinc(II) ions as a component of the artificial AT,[3] the former ions are not suitable in the present bienzyme system due to the following reasons: the transamination mediated by copper(II) ions is too fast to follow the reaction by HPLC, and the LDH activity is strongly inhibited by copper(II) ions.
7 A. Meister, *J. Biol. Chem.*, **184**, 117 (1949).