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A Holoenzyme Model of Thiamin Dependent Enzyme; Asymmetrical Acyloin Condensation Using a Lipid Catalyst in a Bilayer Membrane

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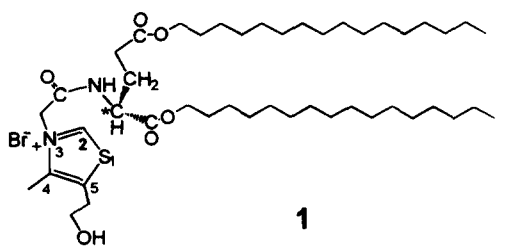
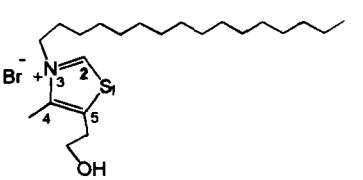
Abstract: Within a DPPC bilayer membrane, the lipid catalyst **1**, having a thiazolium salt unit in the head group, exhibited catalytic activity and enantioselectivity in the acyloin condensation of benzaldehyde. The activity and enantioselectivity were strongly dependent upon the reaction temperature. The lipid bilayer membrane enhanced both the catalytic activity and the enantioselectivity and can be regarded as an apoenzyme model for the thiamin coenzyme.

An apoenzyme not only fixes a coenzyme in place but also furnishes a specific reaction field for enzymatic catalysis.[1] The apoenzyme is also known to provide a degree of protection for the coenzyme.[2] When one is designing an artificial holoenzyme system, it is therefore important to prepare an appropriate apoenzyme. We have focused our attention on a model study of catalysis with a thiamin dependent enzyme.[3,4] The thiazolium salt unit, which is an active center in the coenzyme thiamin pyrophosphate,[5] is known to be decomposed in aqueous solution from attack by hydroxide ions.[6] However, the thiamin coenzyme functions in vivo even in hydrophilic environments because the apoenzyme protects the thiazolium salt unit during the enzymatic reaction. In studies of thiamin dependent holoenzyme models, artificial apoenzymes have been constructed from various molecular assembly systems such as a micelle,[7] a cyclodextrin,[8] a cyclophane,[9] and a vinyl polymer.[3,4] Certainly, these systems provided hydrophobic microenvironments which enhanced the catalytic activity and protected the active site but they did not provide a specific (stereospecificity and/or substrate specificity) field for the catalysis. In contrast, a lipid bilayer membrane should supply such a specific field for the catalysis. Some research groups have previously used this type of system to obtain enantioselective catalytic systems.[10,11] We have designed a new lipid catalyst **1**, which has a thiazolium salt unit in the head group. This thiazolium salt catalyst has an optically active center near its catalytic active site (thiazolium C-2). Previous studies of enantioselective acyloin condensations have been carried out with thiazolium salt catalysts having only an optically active group near a catalytic active site.[12,13] However, the matrix lipids in our system (such as dipalmitoyl *l*-α-phosphatidylcholine, DPPC) are thought to form an optically active field so it is anticipated that the combination of the chiral site and



1



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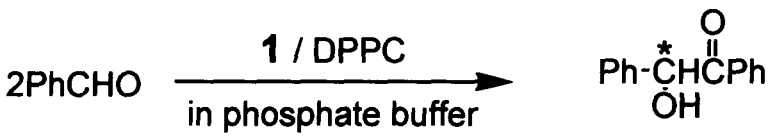

Scheme 1

the neighboring chiral group will synergistically induce enantioselectivity in the catalysis.

The lipid catalyst **1** was prepared from *l*-glutamic acid according to the method described by Kunitake.[14] The structure of the catalyst was confirmed by ^{1}H NMR, elemental analyses, and mass spectrometry.[15] A vesicle solution was prepared by sonication of the lipid thin film in buffer solution using a probe type ultra-sonicator. The lipid bilayer membrane was constructed from 1 molar equivalent of catalyst **1** and 4 molar equivalents of DPPC. The vesicle solution was characterized by DSC and a fluorescence depolarization method. The results indicated that **1** had a clear phase transition temperature at approximately 40 °C and that **1** did not disturb the morphology of the DPPC membrane. The acyloin condensation of benzaldehyde was used to evaluate the catalytic activity of **1** in pH 8.0 phosphate buffer (Scheme 1). The reaction was monitored by using HPLC to measure the amount of benzoin that was generated. The HPLC system (JASCO-TriRoter) consisted of a Chiralpak OP(+) column, a mobile phase of methanol and a UV detector. This method also allowed us to identify both the (+) and (-) enantiomers of benzoin.

The catalytic activity of **1** (35 % benzoin yield) was comparable to that of a surfactant catalyst, N-cetyl-5-hydroxyethyl-4-methylthiazolium bromide **2**[16] (55 % yield), in phosphate buffer (pH 8.0, 0.055 M), in which solvent a low molecular weight analog was found to have no activity.[2,3] Therefore, the lipid catalyst in the bilayer membrane system exhibited an efficiency similar to that observed with the other molecular assembly catalysts formed from micelles[7] or polymers.[2,3] The lower activity of the lipid catalyst is likely a consequence of the rigidity of the membrane. The rigidity of the membrane is actually detrimental to the catalytic activity but may be advantageous in controlling the enantioselectivity of the acyloin condensation. Figure 1 shows the activity and the enantioselectivity of the catalysis as functions of temperature. The catalytic activity was strongly dependent upon the temperature, particularly near the phase transition temperature (Tc; 40 °C). The catalyst exhibited its maximum activity at Tc which is probably a result of increased mobilities for the reacting substances in the membrane. Additionally, it was observed that the enantioselectivity increased as the temperature decreased and that the membrane catalyst produced particularly high e.e. values ((S)-(+)-benzoin excess) below Tc. Temperature did not have the same effect on the behavior of the lipid catalyst in ethanol (Figure 2) and the enantioselectivity was also low (maximum,

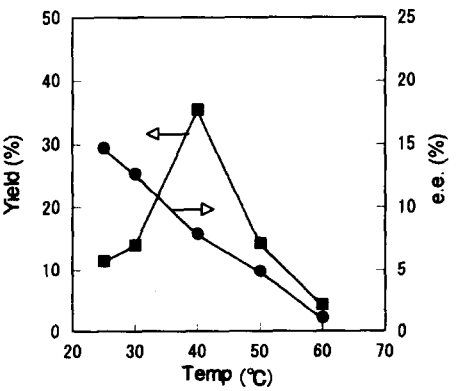

Figure 1 Temperature dependence of catalytic activity (■) and e.e. (●) in benzoin condensation. PhCHO = 0.10 M, **1** = 0.01 M, DPPC = 0.04 M in phosphate buffer (0.055 M, pH 8.0) for 42 h.

5% e.e.). The different e.e. values obtained with the bilayer membrane system and the homogeneous ethanol solution at 25 °C provided evidence that a chiral field present in the DPPC bilayer membrane enhanced the enantioselectivity of the lipid catalyst.

The surfactant catalyst **2** was also utilized as a catalyst in a DPPC bilayer membrane. The activity was influenced by the reaction temperature in a manner similar to that observed with the **1**/DPPC co-vesicle system, as shown in Figure 3. However, in comparison to the lipid catalyst, the enantioselectivity of **2** was low and independent of reaction temperature. This indicates that the chiral field present in the DPPC membrane was not sufficient by itself to produce the enantioselectivity that was observed with catalyst **1**. It is clear that both the chirality of **1** and the chiral field within the bilayer membrane work synergistically to effect the enantioselective acyloin condensation.

In conclusion, the lipid bilayer membrane enhanced the catalytic activity and the enantioselectivity of the lipid catalyst. Therefore, the lipid bilayer membrane system is a potential apoenzyme model and the thiazolium salt lipid catalyst may be used as an artificial thiamin coenzyme.

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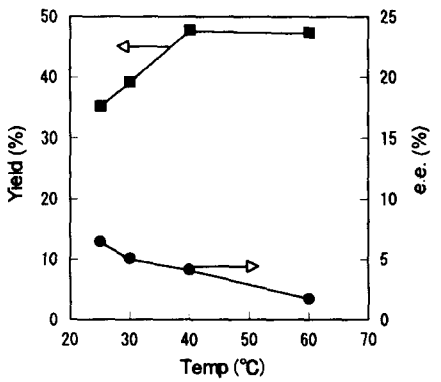

Figure 2 Temperature dependence of catalytic activity (■) and e.e.(●) in ethanol solution. PhCHO = 0.10 M, 1 = 0.01 M, Et_3N = 0.025 M in EtOH for 42 h.

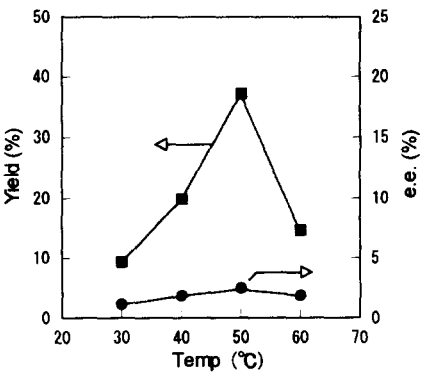

Figure 3 Temperature dependence of catalytic activity (■) and e.e. (●) of surfactant catalyst **2** in benzoin condensation. PhCHO = 0.10 M, **2** = 0.01 M, DPPC = 0.04 M in phosphate buffer (0.055 M, pH 8.0) for 42 h.

References and notes

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15. ^{1}H NMR(200 MHz, $CDCl_3$), δ 0.88(6H, t, $\mathbf{CH_3}(CH_2)_{15}$), 1.25(52H, m, $CH_3(\mathbf{CH_2})_{13}CH_2$), 1.50~1.72(4H, m, $OCH_2\mathbf{CH_2}$), 2.11~2.27(2H, m, $CH\mathbf{CH_2}CH_2$), 2.48~2.57(5H, m, $CHCH_2\mathbf{CH_2}$, $N\mathbf{CH_3}$ <thiazolium>), 3.06(2H, br, $\mathbf{CH_2}CH_2OH$), 3.83(2H, br, $CH_2\mathbf{CH_2}OH$), 4.01~4.11(4H, m, $\mathbf{CH_2}OCO$), 4.42~4.52(1H, m, **CH**), 5.78(2H, s, $CO\mathbf{CH_2}N$), 9.25(1H, d, **NH**), 10.33(1H, s, **thiazolium 2H**).
C. H. N. (%), Calcd; 62.84, 9.73, 3.26, Found; 62.79, 9.80, 3.11, FABMS, m/z 780 (M^+). $[\alpha]_D$=-8.1 (C;2.00, $CHCl_3$).
16. The surfactant catalyst **2** had a critical micellar concentration (cmc) at 4.5×10^{-4} M.

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