Bifunctional Receptors. Rate Accelerations of Oxidative Decarboxylation of Pyruvate by Thymine-Thiazolium in the Presence of a Melamine Derivative Bearing a Guanidinium Ion

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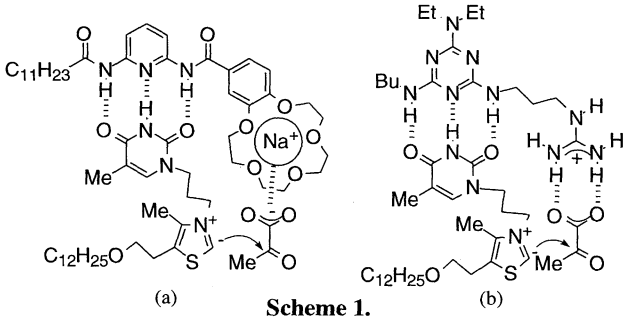
It was found that melamine derivatives bearing a guanidinium ion assemble a thymine-thiazolium ion and pyruvate in close proximity via hydrogen bonds to enhance the rate of the oxidative decarboxylation in $CHCl_3$-MeCN (9 : 1 v/v), where the guanidinium ion not only binds pyruvate but also activates it.

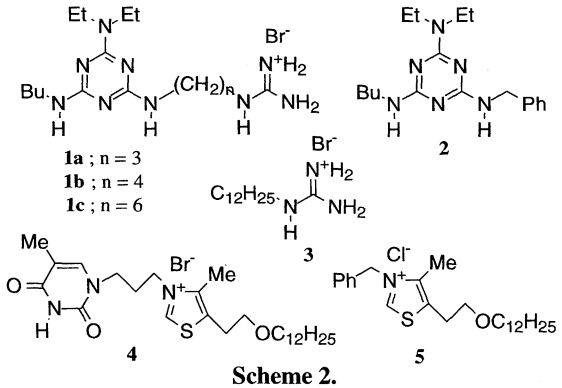
Introduction of apoenzyme functions into catalytic systems is inevitable for construction of artificial enzymes. A number of studies on catalytic systems possessing a substrate-binding site have been reported as enzyme models.[1] For thiamine model systems, a cyclodextrin,[2] a cyclophane,[3] a macrotricyclic quaternary ammonium ion,[4] and a crown ether[5] have been used as a substrate-binding site. The thiazolium ion, however, is covalently connected to these functional groups. We have reported that 2,6-amidopyridine derivative having a benzocrown ether assembles a thymine-thiazolium ion **4** via hydrogen bonds and pyruvate by electrostatic interaction of Na^+ bound into the crown cavity as shown in Scheme 1(a), giving the rate acceleration of the oxidative decarboxylation in $CHCl_3$-MeCN (9 : 1 v/v).[6] This system, however, does not possess the substrate-activating and/or transition state-stabilizing functions. Much larger rate accelerations would be achieved if such functions are introduced into the receptor molecule. Meanwhile, association of carboxylate and a guanidinium ion by hydrogen bonding is



Scheme 1.

well established,[7] and furthermore hydrogen-bonded phenylglyoxylate by an amidinium ion is known to be reduced by an NADH model in MeCN, whereas not for ethyl phenylglyoxylate.[8] This suggests that a guanidinium ion could be used as a pyruvate-binding and -activating moiety. This prompted us to examine rate enhancements due to a noncovalently assembled system bringing with pyruvate activation by the guanidinium ion as shown in Scheme 1(b). The receptors and thiazolium ions employed are shown in Scheme 2.[9] The reactivities of the thiazolium ions were kinetically estimated by employing flavin oxidation of the active aldehyde in $CHCl_3$-MeCN (9 : 1 v/v) containing 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) under anaerobic conditions as described previously.[5,6]

In the first place, the effects of the melamine derivative **2** and guanidinium ion itself **3** on the rates for **4**[6] were examined. As



Scheme 2.

shown in Figure 1, the rate (V_{obs}) increases with the increase of [**3**] to reach a saturation, whereas the rate is not affected by addition of **2**. It was confirmed that the rate acceleration is not observed by addition of cetyltrimethylammonium bromide. It was also confirmed that such a rate acceleration by **3** is not observed in EtOH. These results clearly indicate that pyruvate is activated by the guanidinium ion via hydrogen bonds accompanying charge neutralization. The observed rate (V_{obs}) is a summation of the rate for free pyruvate and that for hydrogen-bonded pyruvate (**3**•pyruvate) in an allotment proportional to the each concentration. To evaluate the concentrations of the each pyruvate, the binding constant of **3**•pyruvate (K_1) was determined to be 3700 M^{-1} (1 M = 1 mol dm^{-3}) in $CDCl_3$ in ^1H NMR titration.[10] This allows us to estimate the amount of **3**•pyruvate to be less than 51% of the total amount of pyruvate under the reaction conditions.[11] Namely the observed rate could be written as $V_{obs} = 0.49\ V_o + 0.51\ V_p$, where V_o and V_p are the rate without **3** and that from **3**•pyruvate, respectively. Since V_o and V_{obs} are 1.40×10^{-8} Ms^{-1} and 1.29×10^{-7} Ms^{-1}, V_p is calculated to be 2.39×10^{-7} Ms^{-1}. Thus, the rate acceleration due to the guanidinium ion **3** is more than 17-fold (V_p/V_o), indicating that the guanidinium ion itself binds and activates pyruvate in the present reaction.

The effect of **1**, which are able to assemble both **4** and pyruvate via hydrogen bonds, was examined similarly. As shown in Figure 2, the rates increase with the increase of [**1**] and the rate accelerations are dependent on the length of the methylene spacer in **1**. At [**1**] = 8.0×10^{-4} M, the apparent rate accelerations are 90-fold for **1a**, 50-fold for **1b**, and 20-fold for **1c**, respectively, indicating that the geometrical positioning of the thiazolium ion and pyruvate in the complex is important for the rate accelerations. In the presence of **1**, coexisting species in the solvent are free **4**, **1**•**4**, free pyruvate, **1**•pyruvate, and **1**•**4**•pyruvate. If it is assumed that the reactivities of free **4** and **1**•**4** are similar and pyruvate activation by the guanidinium moiety of **1** is similar to that by **3**, the rate accelerations due to the ternary complex formation would be estimated as described for

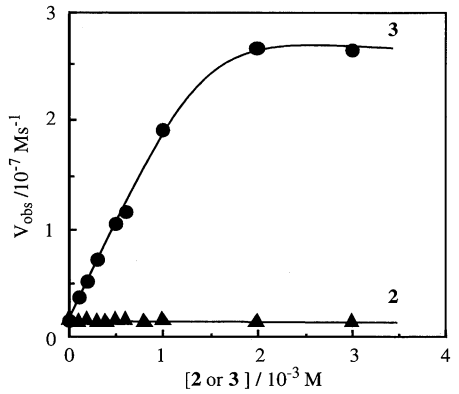


Figure 1. The concentration effect of **2** or **3** on the rates. [**4**] = 2.00 x 10^{-4} M, [$MeCOCO_2H$] = 1.00 x 10^{-3} M, [DBU] = 2.00 x 10^{-3} M, [Flavin] = 2.00 x 10^{-5} M, $CHCl_3$: MeCN = 9 : 1 (v/v), N_2, 25 °C.

3•pyruvate; V_{obs} would be simply represented as a summation of V_o, V_p, and V_c (the rate from **1**•**4**•pyruvate as shown in Scheme 1(b)) in an allotment proportional to the each concentration. Since the binding constant for **1**•**4** was unable to be determined owing to broadening of N-H protons, the binding constant between **2** and 1-(3-bromopropyl)thymine (43 M^{-1} in $CDCl_3$) was used as that for **1**•**4** (K_2),[12] giving the complex formation to be 3% of the total amount of **4** under the reaction conditions. The ratio of V_o, V_p, and V_c in V_{obs} is evaluated to be 0.490 : 0.495 : 0.015 by using K_1 and K_2: V_{obs} = 0.490 V_o + 0.495 V_p + 0.015 V_c. V_c is calculated to be 7.6 x 10^{-5} Ms^{-1} by using V_{obs} = 1.27 x 10^{-7} Ms^{-1}, V_o and V_p obtained for **3**. The rate acceleration due to formation of **1**•**4**•pyruvate is 5 x 10^3-fold (V_c/V_o) for **1a**.[13] Similarly the rate accelerations due to **1b** and **1c** are evaluated to be 3 x 10^3-fold and 1 x 10^3-fold, respectively. In the case of **5**, however, such a rate acceleration except for pyruvate activation was not observed.

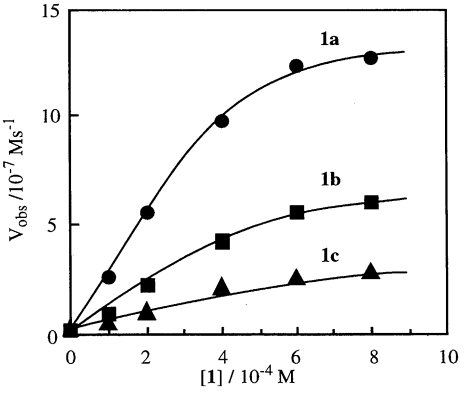


Figure 2. The concentration effect of **1** on the rates. [**4**] = 2.00 x 10^{-4} M, [$MeCOCO_2H$] = 1.00 x 10^{-3} M, [DBU] = 2.00 x 10^{-3} M, [Flavin] = 2.00 x 10^{-5} M, $CHCl_3$: MeCN = 9 : 1 (v/v), N_2, 25 °C.

Although template is a well-known concept,[14] functionalized receptors possessing both binding and activating abilities are scarcely reported in spite of a key building block for construction of artificial enzymes.

In summary, we demonstrated that (i) a guanidinium ion binds and activates pyruvate by hydrogen bonding, and (ii) the melamine derivatives bearing a guanidinium ion binds both the thymine-thiazolium ion and pyruvate in close proximity to bring about large rate accelerations. Such molecules could be regarded to display a function of apoproteins.

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References and Notes

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9 The receptors (**1**) were prepared according to essentially same procedures as described in our previous study; S. Ohshima, N. Tamura, T. Nabeshima, and Y. Yano, *J. Chem. Soc., Chem. Commun.*, 712 (1993). **1a**: mp 141-142 °C ($CHCl_3$-diethyl ether). Found C, 41.2; H, 7.51; N, 29.1%. Calcd for $C_{15}H_{32}BrN_9•H_2O$: C, 41.28; H, 7.85; N, 28.89%. **1b**: mp 138-141 °C ($CHCl_3$-diethyl ether). Found C, 43.8; H, 7.72; N, 28.4%. Calcd for $C_{16}H_{34}BrN_9•0.5H_2O$: C, 43.53; H, 7.99; N, 28.55%. **1c**: pasty solid. Found C, 43.4, H. 8.28, N, 25.5%. Calcd for $C_{18}H_{38}BrN_9•2H_2O$: C, 43.54; H, 8.53; N, 25.39%.

10 The binding constant of **3**•pyruvate (K_1) was determined by recording the chemical shifts of N-H proton of **3** by titration with pyruvic acid and triethylamine in $CDCl_3$.

11 [Pyruvate] = 1.00 x 10^{-3} M, [**3**] = 8.00 x 10^{-4} M. K_1 in $CHCl_3$-MeCN (9 : 1 v/v) is considered to be smaller than that in $CDCl_3$. This means that the amount of **3**•pyruvate in $CHCl_3$-MeCN is less than 51% . Less complex formation results in a larger rate acceleration.

12 The binding constant (K_2) was determined by following the chemical shifts of the thymine N-H by titration with **2** in $CDCl_3$. The binding constants in $CDCl_3$-CD_3CN (9:1 v/v) could not be obtained due to broadening of of N-H protons.

13 Since K_1 and K_2 in the present solvent system are expected to be smaller than those in $CDCl_3$, real rate enhancements should be larger than this value.

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