A Rate-accelerating Noncovalently Assembled System for Thiazolium-catalysed Oxidative Decarboxylation of Pyruvate in Chloroform–Acetonitile

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It is found that a thiazolium ion having a thymine moiety accelerates the rate of the oxidative decarboxylation of pyruvate in the presence of a 2,6-diamidopyridine derivative bearing a crown ether and Na^+ in $CHCl_3$–MeCN (9 : 1 v/v).

To construct artificial enzymes, it is of primary importance to assemble catalytic groups and a substrate in a suitable position by covalent and/or noncovalent bonds.[1] We have previously reported that an alkali metal cation such as Na^+ or K^+ bound in the crown cavity of **1** attracts a pyruvate anion into the vicinity of the catalytic site to bring about the rate acceleration of the oxidative decarboxylation.[2] Hamilton and Engen have shown that a 2,6-diamidopyridine derivative acts as a thymine receptor by the means of three hydrogen bonds in $CHCl_3$.[3] These facts prompted us to design a noncovalently assembled system which involves a thiazolium ion bearing a thymine moiety **2** and a 2,6-diamidopyridine derivative having a crown ether **4** in the presence of an alkali metal ion. Namely **4** forms a molecular complex with **2** *via* three hydrogen bonds, and the alkali metal cation bound into the crown moiety attracts electrostatically a pyruvate anion to form a supramolecular complex, resulting in the rate accelerations. This possibility was tested by employing the compounds **2** and **4** (Scheme 1).

Compound **2** was synthesized from 4-(2-dodecyloxyethyl)-5-methylthiazole and 1-(3-bromopropyl)thymine[4] in MeCN (72%, m.p. 96–8 °C).† Receptor **4** was synthesized by stepwise acylations of 2,6-diaminopyridine in tetrahydrofuran (THF) (32%, m.p. 162–5 °C).‡

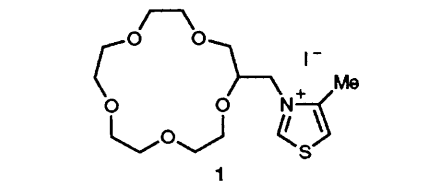
For the noncovalently assembled system *via* both hydrogen bonds and electrostatic forces, choice of solvents is quite important. We chose a solvent system of $CHCl_3$–MeCN (9 : 1 v/v) in which NaI is soluble under the reaction conditions. The association constant of **2** and **4** was determined to be 173 $dm^3\ mol^{-1}$ in $CDCl_3$–CD_3CN (9 : 1 v/v) by ^1H NMR.§

The catalytic activities of the thiazolium ions were estimated kinetically by employing flavin oxidation of the active aldehyde formed from pyruvic acid (PA) 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] Firstly it was confirmed that the rate of the oxidative decarboxylation with **1** is significantly increased on addition of Na^+ (150-fold) in $CHCl_3$–MeCN (9 : 1 v/v) (Fig. 1), whereas only 10-fold in EtOH.[2] This much larger rate-acceleration could be explained by the increase of local concentrations of

† HRMS (fast atom bombardment); Found: 478.3129. Calc. for $C_{26}H_{44}N_3O_3S$ (M^+ – Br) 478.3103.

‡ Compound **1** was supplied from our previous study (ref. 2).

§ *K* was determined from downfield shifts of N–H of **2** by changing the concentration of **4**.



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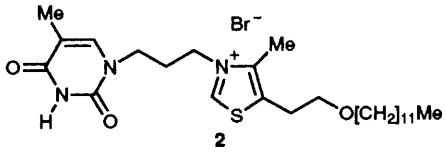

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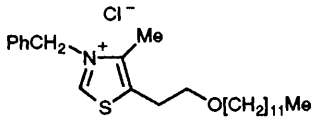

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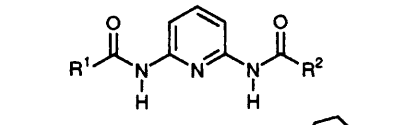
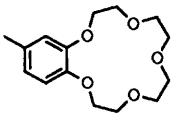


4; $R^1 = [CH_2]_{10}Me$, R^2 =



5; $R^1 = R^2 = Me$

Scheme 1

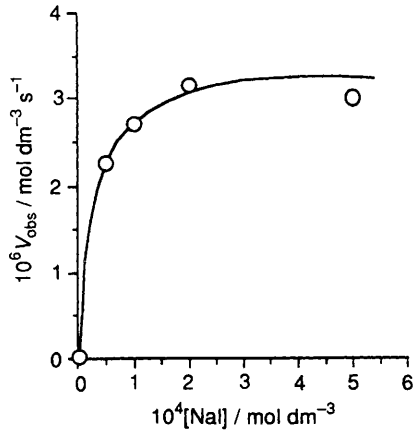


Fig. 1 Plot of V_{obs} *vs.* [NaI]; [**1**] = 1.0 × 10^{-4} mol dm^{-3}, [PA] = 1.0 × 10^{-3} mol dm^{-3}, [DBU] = 2.0 × 10^{-3} mol dm^{-3}, [flavin] = 2.0 × 10^{-5} mol dm^{-3}, 25 °C, N_2, $CHCl_3$–MeCN = 9 : 1 (v/v)

pyruvate due to stronger interaction between a pyruvate anion and Na^+ bound into the crown ring in $CHCl_3$–MeCN.

The effect of Na^+ on the rates of reaction of **2** and **3** is shown in Fig. 2. Fig. 2 shows that the catalytic activities of **2** and **3** were almost the same in the absence of the receptor. In the presence of the receptor **4**, however, the rate of reaction of **2** was increased with the increase of [Na^+] (10-fold at the maximum), whereas no Na^+ effect was observed without **4** or with **5**. The rate-accelerating effect was sensitive to the solvent polarity. Namely no rate acceleration was observed on addition of EtOH (15% v/v) into the present solvent system. It was also confirmed that the rate of reaction of **3** is not affected by addition of Na^+ with or without the receptors **4** and **5**. These observations suggest that the receptor **4** forms a molecular complex with **2** *via* the three hydrogen bonds, in which the crown moiety is located in the proximity of the

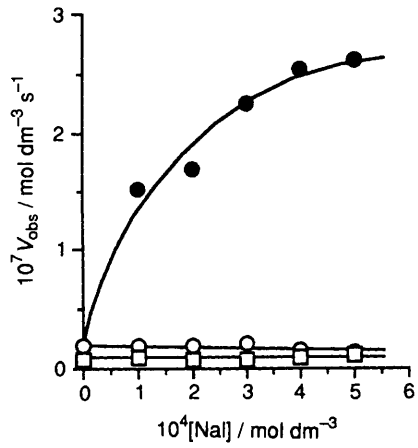
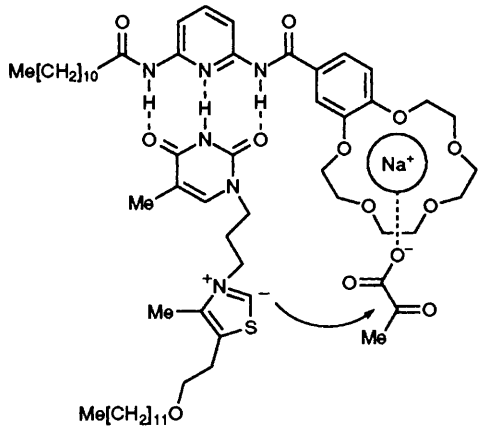


Fig. 2 Plots of V_{obs} *vs.* [NaI]; [PA] = 1.0 × 10^{-3} mol dm^{-3}, [DBU] = 2.0 × 10^{-3} mol dm^{-3}, [flavin] = 2.0 × 10^{-5} mol dm^{-3}, 25 °C, N_2, $CHCl_3$–MeCN = 9 : 1 (v/v): ● [**2**] = 1.0 × 10^{-4} mol dm^{-3}, [**4**] = 5.0 × 10^{-4} mol dm^{-3}; ○ [**2**] = 1.0 × 10^{-4} mol dm^{-3}; □ [**3**] = 1.0 × 10^{-4} mol dm^{-3}, [**4**] = 5.0 × 10^{-4} mol dm^{-3}



Scheme 2

thiazolium ring, and Na^+ bound in the crown cavity attracts a pyruvate anion into the vicinity of the catalytic site as illustrated in Scheme 2.

The rate (V_{obs}) is a summation of rates from the complex (V_c) and the free state (V_o) in an allotment proportional to the concentration of each. The amount of the complex formation of **2** and **4** is estimated to be 8% of the total amount of **2** under the reaction conditions by using K = 173 dm^3 mol^{-1}. V_c is calculated to be 3.0 × 10^{-6} dm^3 mol^{-1} s^{-1} that is 160 times larger than V_o (the rate of **2** without **4**).

The present results demonstrate that the receptor assembles the catalyst and the substrate *via* noncovalent interactions such as hydrogen bonds and electrostatic interactions to provide the rate acceleration. Such a receptor could be regarded as an apoenzyme model.

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