Rate-accelerating Metal Ion Effects on Decarboxylation of α-Keto Acids by a Thiazolium Ion bearing a Metal Binding Site

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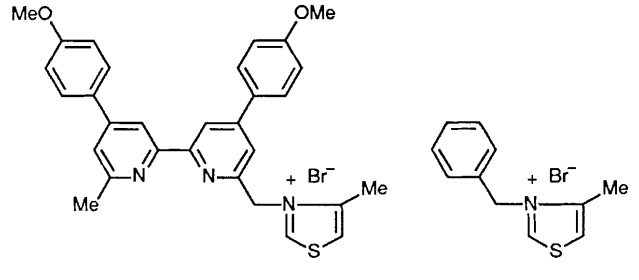
Decarboxylation of α-keto acids by a thiazolium ion bearing a bipyridine moiety was found to be markedly enhanced by divalent metal ions such as Zn^{2+}, Mn^{2+} and Mg^{2+} in EtOH.

Pyruvate decarboxylase (EC 4.1.1.1), which contains thiamine pyrophosphate (TPP) and Mg^{2+} as cofactors, catalyses the decarboxylation of pyruvate to afford acetaldehyde and CO_2 in a multistep mechanism, which has been established to involve: (*i*) deprotonation of the 2-position of TPP, (*ii*) nucleophilic attack of the ylide on pyruvate to form 2-α-lactyl-TPP, (*iii*) decarboxylation of the adduct to form the so called 'active aldehyde' and CO_2, and (*iv*) acetaldehyde release from the active aldehyde.[1] Mg^{2+} ion is thought to be required for the holoenzyme formation as well as the catalytic activity.[2] However, the role of the metal ion in the catalytic activity is not yet clear,[1a] although complex structures of divalent metal ions with TPP[3] and 2-(α-hydroxybenzyl)-TPP,[4] and thiamine-dependent enzyme mimics[5] have been investigated.

We herein report a large rate-accelerating metal ion effect on the decarboxylation of α-keto acids by employing the thiazolium ion **1** covalently bound with a bipyridine moiety as a metal binding site; compound **2** was used for comparison. The catalyst **1** was synthesized from 6-bromomethyl-6′-methyl-4,4′-bis(*p*-methoxyphenyl)-2,2′-bipyridine[6] and 4-methylthiazole.†



1 **2**

† Yield 78%, m.p. 198–200 °C (decomp.) (from MeCN–$CHCl_3$). Satisfactory characterisation data [^{1}H NMR and microanalysis (C, H, N)] were obtained.

The catalytic activity of the thiazolium ions was determined kinetically by employing flavin-oxidation of the active aldehyde formed from the α-keto acid or aldehyde in EtOH containing 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) under anaerobic conditions.[7] Zero-order rate constants (V_{obs}) determined from the absorption decrease of 3,10-dimethylisoalloxazine at 443 nm involve the overall rates of the steps (*i*), (*ii*) and (*iii*).

$RCO{-}CO_2H$
3; R=Me
4; R=Ph

In the absence of metal ions, it was found that the catalytic activities of **1** and **2** towards pyruvic acid **3** are almost the

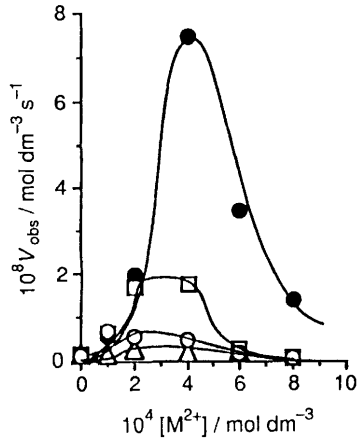


Fig. 1 Plots of V_{obs} *vs.* [M^{2+}]; [**3**] = 1.00 × 10^{-3}, [DBU] = 2.00 × 10^{-3}, [flavin] = 2.5 × 10^{-5} mol dm^{-3}, 25 °C, N_2, EtOH: ● Zn^{2+}, Δ Mg^{2+}, □ Mn^{2+} with [**1**] = 4.00 × 10^{-4} mol dm^{-3}; ○ Zn^{2+} with [**2**] = [6,6′-dimethyl-2,2′-dimethyl-2,2′-bipyridine] = 1.00 × 10^{-4} mol dm^{-3}

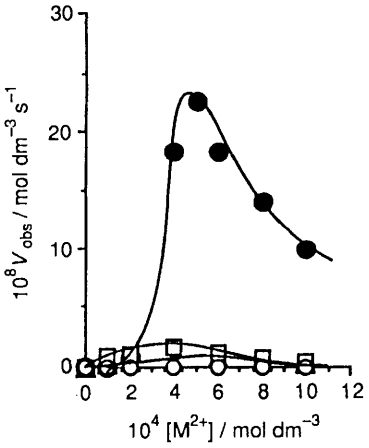
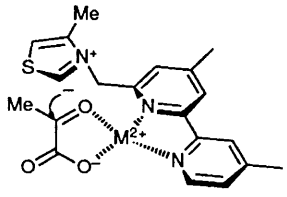


Fig. 2 Plots of V_{obs} *vs.* [M^{2+}]; [**4**] = 1.00 × 10^{-3}, [DBU] = 2.00 × 10^{-3}, [flavin] = 2.5 × 10^{-5} mol dm^{-3}, 25 °C, N_2, EtOH: ● Zn^{2+}, △ Mg^{2+}, □ Mn^{2+} with [**1**] = 4.00 × 10^{-4} mol dm^{-3}; ○ Zn^{2+} with [**2**] = [6,6′-dimethyl-2,2′-bipyridine] = 1.00 × 10^{-4} mol dm^{-3}



Scheme 1 A plausible structure for the reactive complex

same (V_{obs} = 1.76 × 10^{-9} mol dm^{-3} s^{-1} for **1** and 1.17 × 10^{-9} mol dm^{-3} s^{-1} for **2**), and the rates for benzoylformic acid **4** are quite slow (V_{obs} = <1 × 10^{-11} mol dm^{-3} s^{-1} for **1** and **2**) under the conditions of Fig. 1. The lower reactivity of benzoylformic acid **4** could be explained by steric hindrance due to the phenyl group.[8] Addition of metal ions [as $M(NO_3)_2 \cdot 6H_2O$] was found to increase the rates dramatically only when **1** was employed. The rates were quite sensitive to the concentration of the metal ions (Figs. 1 and 2). Maximum rate accelerations are: >10^4-fold (Zn^{2+}), >10^3-fold (Mn^{2+}) and >700-fold (Mg^{2+}) for benzoylformic acid **4** and 43-fold (Zn^{2+}) for pyruvic acid **3**.‡ In contrast, no such rate enhancement was observed when **2** was used.§ Plots of V_{obs} for **1** *vs.* [**3**] with Zn^{2+} gave a saturation curve, whereas a straight line was obtained without Zn^{2+} (not shown), suggesting that the reaction proceeds *via* the ternary **1**–Zn^{2+}–α-keto acid complex shown in Scheme 1. In this complex, the metal ion bound by the bipyridine moiety binds the α-keto acid to facilitate the nucleophilic attack [step (*ii*)] and/or the decarboxylation [step (*iii*)] by acting as a Lewis acid. Complex formation leads to much larger rate enhancements for **4** probably because of relief of the steric hindrance of the phenyl group.

‡ The rates for Cu^{2+}, Ni^{2+} and Co^{2+} could not be determined owing to precipitation during the rate measurements.

§ 6,6′-Dimethyl-2,2′-bipyridine (1 equiv. with respect to **2**) was added.

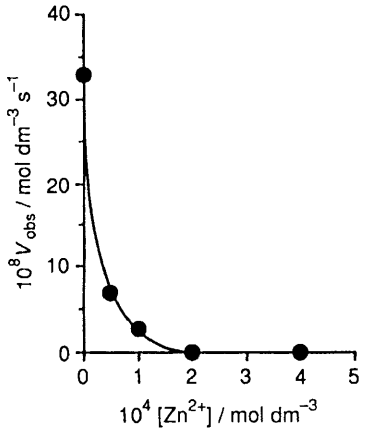


Fig. 3 Plots of V_{obs} *vs.* [Zn^{2+}]; [**5**] = 1.00 × 10^{-3}, [DBU] = 2.00 × 10^{-3}, [flavin] = 2.5 × 10^{-5} mol dm^{-3}, 25 °C, N_2, EtOH

To gain further insight into the metal ion effect, the oxidation of benzaldehyde with **1** and 2-(α-hydroxybenzyl)-3,4-dimethylthiazolium ion[9] by the flavin was kinetically examined in EtOH containing DBU under anaerobic conditions. The rates of both oxidations were decelerated with increasing [Zn^{2+}] (Fig. 3), suggesting that the rate decreases in the presence of excess of metal ions in Figs. 1 and 2 are due to metal inhibition for this step.¶

The present results demonstrate that the metal ion located in the vicinity of the catalytic site binds the α-keto acid to facilitate the steps (*ii*) and/or (*iii*), and also suggest a possible role of the metal ion in thiamine-dependent enzymes. To the best of our knowledge, this is the first example of large rate-accelerating metal ion effect observed for the decarboxylation of α-keto acids in a model system.

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¶ The inhibition may be due to metal-coordination to the hydroxy oxygen and the ring nitrogen of the active aldehyde, reducing the reactivity towards the flavin.