Dimerization of Pyruvate Induced by the Zinc(II)-catalysed Decarboxylation of Oxaloacetate

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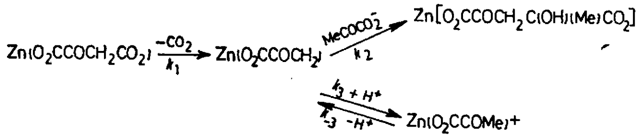
Summary The Zn^{II}-catalysed rate of formation of pyruvate dimer (parapyruvate) is enhanced by the decarboxylation of oxac^{2-}, providing evidence for the postulated existence of intermediate pyruvate enolate in both of these reactions.

The dimerization of pyruvate[1] and the decarboxylation of oxaloacetate (oxac^{2-})[2-6] are catalysed by metalloenzymes and by metal ions alone. Both reactions have been postulated to procede *via* the formation of pyruvate enolate. Direct evidence for the formation of this intermediate has not been provided, and an earlier theory[4,5] that a transient increase in the absorbance of Zn^{II}–oxac^{2-} solutions arises from the formation of a Zn–pyruvate enolate complex [Zn(pyu$_{enolate}$)] has recently been shown to be incorrect.[6] We have now shown that pyruvate is able to trap an intermediate that is formed in the Zn^{II}-catalysed decarboxylation of oxac^{2-}. The product of the trapping is pyruvate dimer [parapyruvate: $^{-}O_2CCOCH_2C(Me)(OH)CO_2^{-}$] Thus, strong confirmatory evidence for the existence of pyruvate enolate as a transient intermediate in these reactions is provided.



Signals arising from the methyl protons of pyruvate and parapyruvate are readily distinguishable in the ^{1}H n.m.r. spectra.[7] A series of solutions in the pH range 3·5—5·5 was prepared containing Zn^{II} and various amounts of oxaloacetate and pyruvate (see legend to Figure). After 30 and 60 min the ^{1}H n.m.r. spectra of the reaction solutions were obtained and the relative amounts of pyruvate and parapyruvate were determined from the areas of the resonance bands. Under the experimental conditions employed oxac^{2-} decarboxylation is essentially complete in 30 min while the normal rate of pyruvate dimerization is much slower (except at pH 5·5). The 30 min spectra showed strong dimer signals. Little additional change was observed in the 60 min spectra except for the same increases in dimer signals that were observed in blanks containing no oxac^{2-}. The excess of dimer formed, after correcting for the blanks, is shown in the Figure as a function of the pyruvate concentration.

The rate of the Zn^{II}-catalysed decarboxylation of oxac^{2-} as measured by u.v. absorption or CO_2 pressure changes[2,4–6] is not appreciably influenced by the presence of pyruvate (except for a slight inhibiting effect arising from the formation of Zn^{II}–pyu$^-$ complexes). Therefore, it is inferred that the induced formation of parapyruvate occurs after Zn(oxac) has lost CO_2 but before the final product, pyruvate, is formed, *i.e.* during the lifetime of some intermediate which, presumably, is Zn(pyu$_{enolate}$).

Both H$^+$ and pyruvate ions compete for Zn(pyu$_{enolate}$) along the paths designated by k_2 and k_3. The independence of the product yield to pH indicates that the rate laws for the competing paths are the same order in H$^+$ concentration, but this order is not defined by the data. The path leading to dimer is dependent on pyruvate concentration, as expected, but the downward curvature and approach to a constant yield seen in the Figure suggest that condensation occurs *via* the formation of a mixed complex, reaction (1).

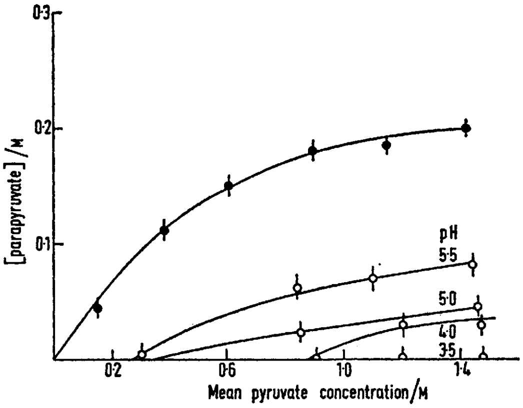


FIGURE. Yield of parapyruvate produced after 30 min at ambient temperature as a function of the mean pyruvate concentration. ● Concentration of parapyruvate formed from 30 solutions initially containing 0·024M $ZnCl_2$, 0·30M oxaloacetate, and 0, 0·3, 0·6, 0·9, 1·2, or 1·5M sodium pyruvate in solutions buffered at pH 3·5, 4·0, 4·5, 5·0, or 5·5. The concentration of dimer formed in the corresponding Zn^{II}–pyruvate blank was subtracted from each data point. Within experimental error all the points for a variation of pH at a given pyruvate level coincided. ○ Concentration of parapyruvate formed in blanks initially containing 0·024M $ZnCl_2$ and 0, 0·3, 0·6, 0·9, 1·2, or 1·5M sodium pyruvate in solutions buffered at pH 3·5, 4·0, 5·0, and 5·5.

$$\mathrm{Zn(pyu_{enolate})} + \mathrm{pyu}^- \underset{\beta_1}{\overset{\text{fast}}{\rightleftharpoons}} \mathrm{Zn(pyu_{enolate})(pyu)}^- \underset{\mathrm{H}^+}{\overset{\text{slow}}{\longrightarrow}} \mathrm{Zn(parapyruvate)} \qquad (1)$$

A value of β_1 in the range 2—3 l mol^{-1} would account for the downward curvature shown in the Figure. This value is consistent with the value of 7 l mol^{-1} reported,[1] for slightly different conditions, for the stepwise constant of the reaction $\mathrm{Zn(pyu^+) + pyu^- \rightleftharpoons Zn(pyu)_2}$. Probably formation of the mixed complex reduces the order of the reaction from second to first, *i.e.* the metal ion promotes the reaction through a proximity effect.

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