Carbon Isotope Effects on the Metal Ion Catalyzed Decarboxylation of Oxalacetate†

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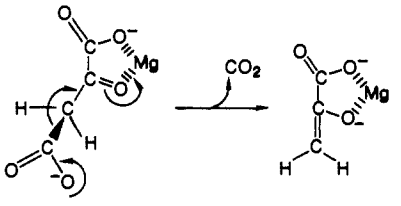
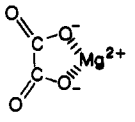
Abstract: The ^{13}C isotope effect on the metal ion catalyzed decarboxylation of oxalacetate has been measured at 25 °C and pH 5.2: Mg^{2+}, 1.0489; Mn^{2+}, 1.0505; Ni^{2+}, 1.044; Cd^{2+}, 1.0492; Zn^{2+}, 1.0504; Co^{2+}, 1.0480. The transition state for decarboxylation appears not to change significantly with a change in divalent metal cation. A ^{13}C isotope effect of 1.038 is observed for the non-metal-catalyzed decarboxylation of monoanionic oxalacetate, indicating an earlier transition state than in the metal ion catalyzed reaction.

Divalent metal cations are thought to play an important role in the enzyme-catalyzed decarboxylation of β-keto carboxylic acids by polarizing the ketone carbonyl, thus creating an electron sink to facilitate decarboxylation.[1-3] Westheimer first examined this question and proposed the following generally accepted mechanism for metal-assisted decarboxylation:



I II

Gelles[3] determined the kinetic constants for decarboxylation of oxalacetate with various metal ions and showed they were linearly related to the stability constant for the analogous metal–*oxalate* complex (III). This supports Westheimer's mechanism if the



III

metal–oxalate complex is considered to be similar in structure to the bidentate metal-oxalacetate transition state of I.

In early studies of decarboxylation reactions, Westheimer reported a primary ^{13}C kinetic isotope effect ($^{12}k/^{13}k$) on decarboxylation of 1.06 with Mn^{2+} at 10 °C and pH 5.9.[2] This value is often quoted as a benchmark by which to gauge the relative size of other isotope effects on decarboxylation reactions. Wood later determined isotope effects of 1.035, 1.034, 1.036, and 1.045 for the Gd^{3+}-, Dy^{3+}-, Y^{3+}-, and non-metal-catalyzed reactions at 25 °C and pH 1.02.[4] There has been a paucity of information on divalent metal cations of biological interest under relevant conditions (pH 7, 25 °C). Hence, this study was undertaken to determine the primary ^{13}C kinetic isotope effect on the decarboxylation of oxalacetate with six divalent metal cations that are known to serve as cofactors for malic enzyme, isocitrate dehydrogenase, and 6-phosphogluconate dehydrogenase, enzymes that catalyze reactions involving the metal-dependent decarboxylation of a 2-keto carboxylic acid.[5-7]

Results and Discussion

The observed ^{13}C primary kinetic isotope effects and rate constants for the decarboxylation of OAA under the stated conditions are shown in Table I. Little change is seen in the $^{12}k/^{13}k$ ratio for decarboxylation of dianionic OAA by the six divalent metal cations examined. The average value is 1.049. The prima facie conclusion is that the transition state for decarboxylation is virtually independent of the metal ion even though the stability constants for the metal–OAA[8] and metal–oxalate complexes[9] change by 2 orders of magnitude throughout this series of metal ions.

Table I. ^{13}C Isotope Effects on Oxalacetate Decarboxylation at 25 °C[a]

	concn, mM	k,[b] min^{-1}	$^{12}k/^{13}k$
		Metal Ion	
Mg^{2+}	10	0.30	1.0489 ± 0.0001
Mn^{2+}	3	0.40	1.0505 ± 0.0001
Ni^{2+}	0.8	2.3	1.044 ± 0.001
Cd^{2+}	1.5	0.42	1.0492 ± 0.0001
Zn^{2+}	0.8	1.7	1.0504 ± 0.0008
Co^{2+}	0.8	1.3	1.0480 ± 0.0001
		No Metal	
monoanionic OAA, pH 2.25		0.20	1.038 ± 0.001
dianionic OAA, pH 7.5		0.022	1.052 ± 0.001[c]

[a] The initial pH was 5.2 unless otherwise noted. Each value is the average of two determinations with fractional reaction 3–7%, compared to four replicates in which decarboxylation was allowed to reach completion. $^{13}C/^{12}C$ ratios were determined with a Finnigan Delta-E isotope ratio mass spectrometer. [b] Values in the presence of metal ions are calculated for the metal ion–OAA complex. [c] Data obtained by Dr. P. V. Attwood in this laboratory.

An explanation consistent with the invariance of the transition state has recently been advanced by Leussing.[10] His application of Marcus' theory[11] to the decarboxylation of OAA estimates $G_0^{\ddagger}$ for the transition state to be 19, 18, and 19 kcal/mol for the Mg^{2+}-, Zn^{2+}-, and non-metal-catalyzed decarboxylation of dianionic OAA, respectively. Leussing concludes that the rate enhancement by metal ions can largely be attributed to an alteration of the thermodynamic distribution of OAA between keto and enol forms. Our data for the metal-catalyzed decarboxylation of dianionic OAA agree with this conclusion. A structure for the transition

† Supported by NIH Grant GM18938.

(1) Abbreviations used: OAA, oxalacetic acid; Mes, 2-morpholinoethane sulfonic acid; DPNH, diphosphopyridine nucleotide, reduced.

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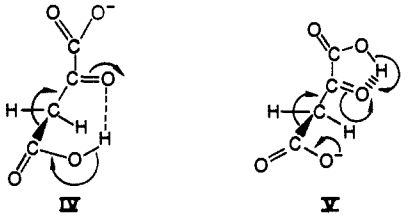
state consistent with this data is I.

The observed ^{13}C isotope effect for non-metal-catalyzed decarboxylation of monoanionic oxalacetate is significantly smaller than any of the metal ion catalyzed values. This suggests an earlier transition state with a more reactant-like structure. Two structures can be suggested for the transition state for the acid-catalyzed process:



IV V

Structure IV will predominate in solution because the pK of the 4-COOH is higher than that of the 1-COOH. However, the hydrogen bonding in this structure tends to flatten the six-membered ring and place C-4 in the plane of the other carbon atoms where it cannot decarboxylate. The fraction of the OAA population occupying protonation state V would be small, but the geometry would be more favorable for decarboxylation. The very slow decarboxylation of fully protonated OAA, which presumably involves IV with the 1-carboxyl protonated,[12] supports the idea that IV is not the major form of the monoanion that decarboxylates (while the proportion of keto form decreases in fully protonated OAA to about 12% of the total, with the remainder being hydrate and enol, neither one of which can decarboxylate,[13,14] the monoanion still decarboxylates 8 times faster than the neutral species after correction for the level of keto form present).

There is no evidence in our experiments for metal ions interacting with monoanionic OAA to promote decarboxylation at a rate faster than the rate with either metal ion or a proton present individually.[15] Hence, the observed rate of decarboxylation of monobasic OAA in the presence of divalent metal cations is the sum of the independent metal-catalyzed and nonmetal-catalyzed reactions.

Experimental Section

^{13}C kinetic isotope effects were determined by the general method of O'Leary,[16] using oxalacetate with 1.1% natural-abundance ^{13}C. The metal chloride salts used in this study were Gold Label quality from Aldrich, and solutions were prepared with water demetaled by successive extraction with dithizone in $CHCl_3$, followed by washes with CCl_4 and aspiration to remove the organic solvent. The solutions were stored in poly(ethylene) bottles washed with concentrated nitric acid. The EDTA used was exhaustively extracted with dithizone according to the above procedure.

CO_2-free OAA solutions were prepared fresh each day by dissolving the solid in demetaled H_2O (extracted with dithizone in $CHCl_3$) at 0 °C and raising the pH to either 2.25 or 5.8 with CO_2-free saturated NaOH. The solutions were sparged with N_2 at 0 °C for 3 h. A 20-mL aliquot of the aqueous metal ion solution containing 1 mM potassium acetate buffer at pH 5 was sparged separately with nitrogen for 4 h. To start the reaction, the appropriate amount of cold OAA solution (typically 1 mL of 0.4 M) was added to the metal ion solution. The initial pH of the reaction medium was determined by the pH of the OAA solution, and the OAA served as the reaction buffer. For those reactions starting above pK_2 (3.85) of OAA, the pH rises during the course of the reaction because H^+ from solution is required to protonate the resulting pyruvate enolate. The reaction mechanism does *not* change as long as the initial pH is sufficiently above pK_2 so that the predominant species in solution is dianionic OAA. Those reactions examining decarboxylation of the monoprotic OAA species were started by the addition of stock OAA solution at pK_1 (2.25). The OAA served as the reaction buffer. The reaction pH should not change during decarboxylation of the monobasic OAA, because the extra proton is available to protonate the pyruvate enolate.

For the metal ion catalyzed decarboxylation of OAA, reaction times were kept under 4 min to minimize the contribution from the nonmetal-catalyzed reaction. The reaction was quenched by the method of Attwood.[17] This involves the injection and rapid mixing of 2 mL of concentrated H_2SO_4 to fully protonate the OAA, which decarboxylates at 25 °C with a rate constant of 3.6×10^{-3} min^{-1} when fully protonated. The reaction vessels were kept at 0 °C to further inhibit decarboxylation until isolation of the CO_2 (typically 1–3 h). The $^{12}C/^{13}C$ ratio of the CO_2 was analyzed on a Finnigan Delta-E isotope ratio mass spectrometer equipped with a dual-inlet system.

The apparent rate of decarboxylation was determined as a function of pH by spectrophotometrically following the disappearance of OAA at 280 nm. The following demetaled buffers were used at 30 mM final concentration: HCl, pH 0–1.5; H_3PO_4, pH 1.5–2.4; tartrate, pH 2.4–4.0; acetate, pH 4.0–5.8; Mes, pH 5.5–7.0. The exact concentration of the OAA solution was determined by enzymatic end point assay with malate dehydrogenase and DPNH. The apparent extinction coefficient of OAA under the specific assay condition was determined by adding a known amount of OAA to a cuvette containing the same buffer solution used in the actual assay and measuring the change in absorbance at 280 nm. The rate of decarboxylation was treated as a first-order process.

Acknowledgment. The NSF provided a major portion of the funding to purchase the isotope ratio mass spectrometer (Grant PCM 8218027). We thank Dr. M. H. O'Leary for helpful discussions.

Registry No. Mg^{2+}, 22537-22-0; Mn^{2+}, 16397-91-4; Ni^{2+}, 14701-22-5; Cd^{2+}, 22537-48-0; Zn^{2+}, 23713-49-7; Co^{2+}, 22541-53-3; ^{13}C, 14762-74-4; monoanionic oxalacetate, 103422-51-1.

(12) Wood[4] determined the ^{13}C isotope effect on OAA decarboxylation at pH 1.02, 25 °C, as 1.0448. This value should represent reaction of the fully protonated molecule, and the higher isotope effect than the one we have observed for the monoanion supports the idea of protonated IV as the reactive species, so that the transition-state structure would differ from that with the monoanion (apparently being later).

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(15) The apparent rate of decarboxylation with divalent Mg^{2+} and Mn^{2+} was determined at pH 7.0 and 2.25. In each case, the apparent rate of decarboxylation with the same level of metal present was at least 10-fold lower at the monoprotic pH than at the dibasic pH.

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