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Kinetic Challenges Facing Oxalate, Malonate, Acetoacetate, and Oxaloacetate Decarboxylases

Richard Wolfenden,* Charles A. Lewis, Jr., and Yang Yuan

Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, North Carolina 27599-7260, United States

Supporting Information

ABSTRACT: To compare the powers of the corresponding enzymes as catalysts, the rates of uncatalyzed decarboxylation of several aliphatic acids (oxalate, malonate, acetoacetate, and oxaloacetate) were determined at elevated temperatures and extrapolated to 25 °C. In the extreme case of oxalate, the rate of the uncatalyzed reaction at pH 4.2 was 1.1×10^{-12} s^{-1}, implying a 2.5×10^{13}-fold rate enhancement by oxalate decarboxylase. Whereas the enzymatic decarboxylation of oxalate requires O_2 and Mn^{II}, the uncatalyzed reaction is unaffected by the presence of these cofactors and appears to proceed by heterolytic elimination of CO_2.

To be useful at the limited concentrations at which they are present within cells (<10^{-4} M),[1] enzymes must act rapidly on their substrates. But in the absence of enzymes, biological reactions proceed with half-lives ranging from <1 min for the dehydration of bicarbonate[2] to >1 billion years for the decarboxylation of glycine.[3] Those rate enhancements are of interest in estimating the power of enzymes and artificial catalysts and their expected sensitivities to transition state analogue inhibitors. Here we compare the rates of spontaneous and enzymatic decarboxylation of oxalate with those of malonate, acetoacetate, and oxaloacetate.

Kinetic experiments on the monoanions of malonate, acetoacetate, and oxaloacetate were conducted in potassium phosphate buffer (pH 6.8), where the corresponding decarboxylases are maximally active.[4−6] The nonenzymatic decarboxylation of oxalate was examined in potassium acetate buffer (pH 4.2), because oxalate decarboxylase is maximally active near pH 4.2.[7] Phosphate and acetate buffers were chosen because acetic acid and the phosphoric acid monoanion (like the acids undergoing decarboxylation) exhibit near-zero (<1 kcal/mol) heats of proton dissociation,[8] canceling the effects of varying temperature on the state of ionization of each substrate. Samples of the potassium salt of each acid (0.01 M) in potassium acetate or phosphate buffer (0.1 M) were introduced into quartz tubes, sealed under vacuum, and placed in convection ovens for various intervals at temperatures maintained within ±1.5 °C as indicated by ASTM thermometers. For each acid, the range of temperatures examined is indicated in Table 1. After the samples were cooled, they were diluted with D_2O containing pyrazine (5×10^{-4} M), which was added as an integration standard. In each case, ^1H NMR analysis showed quantitative conversion of the carboxylic acid to the expected product of decarboxylation. The rates of decarboxylation of malonate and acetoacetate were estimated by monitoring the disappearance of the reactants, and each reaction followed simple first-order kinetics to completion. In the cases of oxaloacetate (whose C−H protons exchange rapidly with solvent water) and oxalate (with no carbon-bound protons), the rates were estimated by monitoring the appearance of the corresponding decarboxylation products, pyruvate and formate. At each temperature, the heating times (between 2 and 72 h) were chosen to allow consumption of the reactant to reach between 15 and 85% completion, yielding individual rate constants with estimated errors of ±3%. These rate constants plotted as a logarithmic function of $1/T$ (with T in K) showed a linear relationship over the full range of temperatures examined, which was used to estimate the enthalpy of activation ($\Delta H^{\ddagger}$) and the rate constant for each uncatalyzed reaction at 25 °C (k_{non}). The results are shown in Table 1, and they are included in further detail in the Supporting Information along with values previously reported for these and other decarboxylation reactions.

Table 1. Rate Constants at 25 °C (k_{non}, s^{-1}) and Thermodynamic Parameters of Activation (kcal/mol) for the Uncatalyzed Decarboxylation of Oxaloacetate, Acetoacetate, and Malonate at pH 6.8 and Oxalate at pH 4.2; Values for the Pure Monoanions Are Given in Italics (Also See the Supporting Information)

reactant (product)	k_{non}	$\Delta G^{\ddagger}$	$\Delta H^{\ddagger}$	$T\Delta S^{\ddagger}$	T range (°C)
oxaloacetate (pyruvate)	2.8×10^{-5}	23.6	17.2	−6.4	23−70
	9.2×10^{-5}	*24.2*	*17.2*	*−7.0*	
acetoacetate (acetone)	3.0×10^{-7}	26.2	23.5	−2.7	23−61
	3.0×10^{-7}	*26.2*	*23.5*	*−2.7*	
malonate (acetate)	1.2×10^{-10}	30.9	30.0	−0.9	80−130
	1.5×10^{-9}	*29.4*	*30.0*	*+0.6*	
oxalate (formate)	1.1×10^{-12}	33.7	26.9	−6.8	150−190
	1.8×10^{-12}	*33.3*	*26.9*	*−6.6*	

The observed rate constants for the decarboxylation of the monoanions of oxaloacetic, acetoacetic acid, and malonic acid monoanions fall close to a linear Brønsted plot based on the pK_a values of the carbon acids produced by decarboxylation (Figure 1), yielding a slope ($\beta = -0.7$) consistent with the development of substantial negative charge at the site where CO_2 elimination occurs.

Enzymes use various strategies to catalyze these decarboxylation reactions, employing an imine-forming lysine residue in the case of acetoacetate or a divalent cation (Mg^{II}, Mn^{II}, Zn^{II}, or Co^{II})

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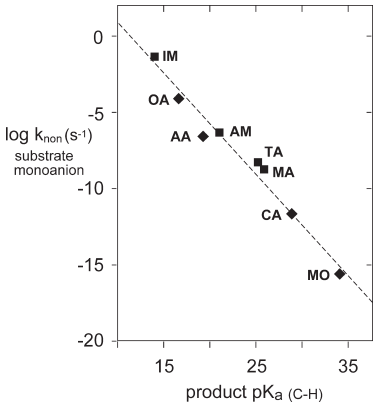

Figure 1. Rate constants at pH 6.8 and 25 °C for decarboxylation of the monoanions of iminomalonate (IM), oxaloacetate (OA), aminomalonate (AM),[15] acetoacetate (AA), trichloroacetate (TA),[16] malonate (MA), cyanoacetate (CA),[17] glycine (GL),[3] and 1-methylorotate (MeO)[18] plotted as a logarithmic function of the pK_a values of the products of their decarboxylation as carbon acids (also see the Supporting Information).

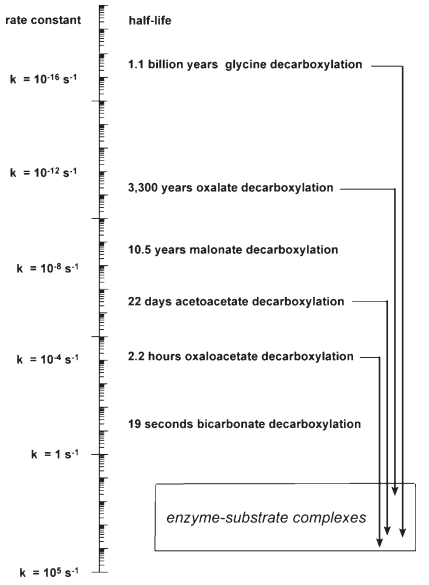

Figure 2. Rate constants and half-lives at 25 °C for decarboxylation of glycine,[3] malonate, acetoacetate, and oxaloacetate at pH 6.8 and oxalate at pH 4.2 in the presence (k_{cat}) and absence (k_{non}) of the corresponding decarboxylases.

in the case of oxaloacetate, whose intervention would be expected to stabilize a transition state with carbanionic character. In the absence of enzymes, those reactions are catalyzed by amines[9] and divalent cations,[10] respectively. The enzymatic elimination of CO_2 from malonate is a more complex process involving preliminary formation of a malonyl—enzyme thioester that appears to be the species that actually undergoes decarboxylation.[5] Oxalate decarboxylase catalyzes a relatively difficult reaction (Table 1) using both a divalent cation (Mn^{II}) and molecular oxygen as cofactors,[11,12] as noted below. Amino acid decarboxylations generally involve transimination of enzyme-bound pyridoxal phosphate (PLP) or a pyruvoyl enzyme, and PLP by itself has been shown to act as an effective catalyst.[3,13]

Figure 2 shows that the k_{cat} values of these enzymes fall within a relatively narrow range (see the Supporting Information); however, because of major differences in the rates of the uncatalyzed reactions, these enzymes vary greatly in the rate enhancements (k_{cat}/k_{non}) that they produce. Particularly striking is oxalate decarboxylase from *Bacillus subtilis*, which is maximally active near pH 4.2 (the pK_a value of the oxalic acid monoanion), where it exhibits a k_{cat} value of 28 s^{-1} (per Mn atom)[7] and generates a rate enhancement of 2.5×10^{13} (Table 2).

The low intrinsic reactivity of oxalate seems understandable in view of the absence of electron-withdrawing groups that might facilitate CO_2 elimination. It is therefore not surprising that oxalate decarboxylase has evolved a special strategy for catalyzing this difficult reaction. The action of oxalate decarboxylase has been shown to involve a radical mechanism in which O_2, an essential cofactor, combines with Mn^{II} in such a way as to permit single electron transfers that facilitate cleavage of the bound oxalate without requiring the formation of a formyl dianion as a discrete intermediate.[14,19] In the cases of oxaloacetate and acetoacetate decarboxylation, amines and metal ion cofactors have been shown to act as catalysts in the absence of the apoenzyme. However, experiments at elevated temperatures (150—200 °C) show that the rate of nonenzymatic decarboxylation of oxalate is not enhanced significantly in solutions to which manganese sulfate (1 M) has been added or by the further addition of oxygen (1×10^{-3} M) or hydrogen peroxide (1 M). Thus, the decarboxylation of oxalate appears to proceed by entirely different mechanisms in the presence and absence of enzyme.

Table 2. Rate Enhancements Produced by Decarboxylases at 25 °C

reactant	pH	k_{non} (s^{-1})	k_{cat} (s^{-1})	k_{cat}/k_{non}
oxaloacetate	6.8	2.8×10^{-5}	7.5×10^{3} [a]	2.7×10^{8}
acetoacetate	6.8	3.0×10^{-7}	1.6×10^{3} [b]	5.3×10^{9}
oxalate	4.2	1.1×10^{-12}	28[c]	2.5×10^{13}
glycine[d]	6.8	2.0×10^{-17}	1.4×10^{3}	7×10^{19}

[a] Taken from ref 6. [b] Taken from ref 4. [c] Taken from ref 7. [d] Taken from ref 3.

■ ASSOCIATED CONTENT

Supporting Information. Present and previously reported rate constants for the nonenzymatic decarboxylation of oxaloacetate, acetoacetate, malonate, and oxalate; pK_a values of carbon acids produced by decarboxylation; and properties of enzymes catalyzing the decarboxylation of oxaloacetate, acetoacetate, malonate, and oxalate. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
water@med.unc.edu

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