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Internal Return of Carbon Dioxide in Decarboxylation: Catalysis of Separation and $^{12}C/^{13}C$ Kinetic Isotope Effects

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Decarboxylation reactions of 2-ketoacids and related compounds have commonly been formulated as occurring in a single irreversible step: C−C bond breaking[1] and rehybridization[2] produces a molecule of carbon dioxide and a carbanion that rapidly separate. However, the major barrier to the reverse reaction, carboxylation of a carbanion, has been presented as being largely entropic,[3,4] with addition of the carbanion to an adjacent molecule of carbon dioxide having a small enthalpic barrier. Application of microscopic reversibility requires that in decarboxylation reactions, carbon dioxide that forms adjacent to the accompanying carbanion (or equivalent) must be subject to attack by the carbanion. This is summarized by the steady-state expression (eq 1) for k_{obs}, the observed rate constant corresponding to Scheme 1, in which the bond-breaking step is associated with k_1 and separation of the products with k_2:

$$k_{obs} = \frac{k_1 k_2}{(k_{-1} + k_2)} \quad (1)$$

The rate constant for C−C bond cleavage, k_1, is subject to a significant intrinsic primary $^{12}C/^{13}C$ kinetic isotope effect (CKIE), enriching the initially formed CO_2 in ^{12}C. However, because of the existence of the reversion step (k_{-1}), the observed CKIE should be smaller than the intrinsic CKIE. An important consequence of this mechanism is its prediction that because k_2 is partially rate-limiting, a catalyst that blocks the reverse reaction should increase the magnitude of the observed CKIE. We have now tested and verified this prediction.

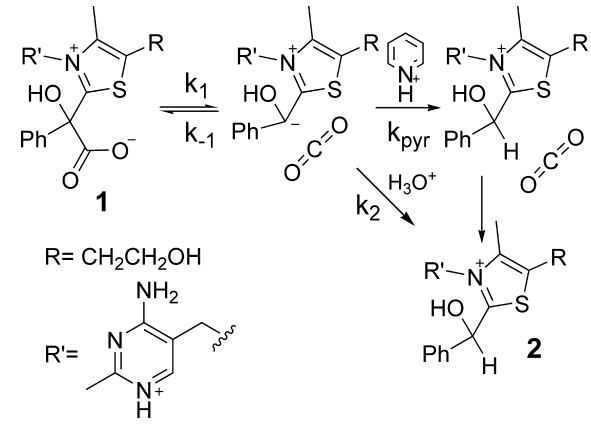
It has been noted that the decarboxylation of mandelylthiamin [MTh (**1**), Scheme 1], a model of the intermediate in benzoylformate decarboxylase, is subject to catalysis by protonated pyridine and its C-alkyl analogues but not by other Brønsted acids.[5,6] It was proposed that this is the result of initial π-stacking of protonated pyridine with MTh. This would enable spectator catalysis[7] by the proton donor that facilitates the separation process via protonation of the nascent carbanion (enamine), permitting CO_2 to separate at a greater net rate: protonated pyridine increases the rate of product formation without affecting the rate constants for C−C cleavage and diffusion. Thus, one observable consequence should be an increase in the magnitude of the observed CKIE on the observed rate constant for decarboxylation of MTh in the presence of the catalyst, with the size of the increase dependent on the extent that $(k_2 + k_{pyr}[PyrH^+])$ is greater than k_2 in eq 2:

$$k_{obs} = \frac{k_1(k_2 + k_{pyr}[PyrH^+])}{k_{-1} + k_2 + k_{pyr}[PyrH^+]} \quad (2)$$

We show the catalysis by pyridinium as a second-order process because we do not know the association constant for the complex of pyridinium with MTh.

On the basis of these considerations, we investigated the effects of pyridinium catalysis on the CKIE for decarboxylation of mandelylthiamin.[8] Measurements were carried out with dissolved MTh (without isotopic enrichment) in a vial with a side arm connected to a digital pressure gauge. CO_2 in the headspace was collected using a pressure-lock syringe and analyzed directly.[9] Data were collected with an isotope-ratio mass spectrometer coupled to a gas chromatograph and combustion interface (GC−IRMS).[10] This required substantially less reactant than classical methods involving isolation of CO_2 and dual-inlet IRMS.[11] Samples were obtained throughout the course of the reaction, and measurements for completed reactions were taken after 24 h. From the changes in pressure and isotope ratio, we determined both the reaction progress and the CKIE.

Scheme 1. Decarboxylation of Mandelylthiamin (**1**) to Hydroxybenzylthiamin (**2**), Showing the Role of Pyridine in Capturing the Carbanion



Bigeleisen's equation,[12] which predicts the isotopic composition of the cumulative CO_2 sampled at any fraction of conversion, f, was used to fit our data (Figure 1). It is well-established that the magnitude of the error in the measured CKIE increases with the extent of the reaction and that this error is minimized by working at low conversion.[13] We measured precise isotopic ratios throughout the course of the reaction; however, we performed CKIE calculations only up to 15% conversion.

We measured $pK_A' = 5.0$ for the conjugate acid of pyridine under our reaction conditions. The reactivity of MTh is greatest where

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its pyrimidine group is protonated and the carboxyl group is dissociated.[8] We examined the reaction rates and CKIE at pH 4 for a solution of protonated pyridine in acetate buffer. The observed first-order rate constants were determined from fitting data obtained from the change in pressure during the course of the reaction. The rates were verified by following the decrease in absorbance from the reactant at 295 nm. Under these conditions, hydroxybenzylthiamin (**2** in Scheme 1) and CO_2 are the exclusive products.[14]

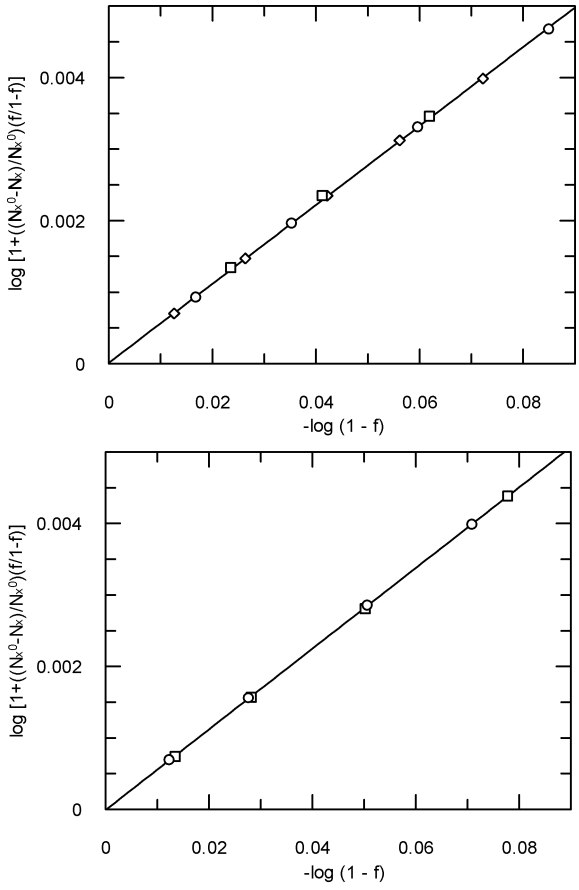

Figure 1. Fits of GC-IRMS data to the Bigeleisen equation, giving the CKIE for decarboxylation of MTh as (top) 1.058 ± 0.0005 in acetate buffer (0.4 M, pH 4, $I = 1$, 25 °C) and (bottom) 1.060 ± 0.0005 in 1 M protonated pyridine buffered in 0.3 M acetate (pH 4, $I = 1$, 25 °C). Individual runs are denoted by different symbols.

The observed first-order rate constant for the decarboxylation in acetate buffer is $(6.5 \pm 0.3) \times 10^{-4}$ s^{-1}. The CKIE for the uncatalyzed reaction (Figure 1) is 1.058 ± 0.0005. In the presence of 1.0 M protonated pyridine catalyst, the rate increases to $(8.8 \pm 0.2) \times 10^{-4}$ s^{-1} and the CKIE increases to 1.060 ± 0.0005. The difference in CKIE (4%) is significant and well outside the range of experimental uncertainty (1%). Catalyst concentrations were maintained at the lowest levels possible in order to minimize perturbation of the polarity of the solvent while still providing a significant shift in the CKIE.[15,16] This necessarily leads to the greater portion of the conversion of MTh proceeding via the uncatalyzed route. Therefore, the intrinsic value of the CKIE for the catalyzed process is blended with that of the uncatalyzed pathway. If we assume that the increase in the observed isotope effect ($\Delta CKIE_{obs} = 0.002$) is proportional to the extent that the reaction proceeds via the catalyzed pathway, the intrinsic isotope effect can be approximated by the inverse of the rate increase: $\Delta CKIE \approx \Delta CKIE_{obs} \times [(k_1(k_2 + k_{pyr}[PyrH^+])/(k_{-1} + k_2 + k_{pyr}[PyrH^+])]/\{[k_1(k_2 + k_{pyr}[PyrH^+])/(k_{-1} + k_2 + k_{pyr}[PyrH^+])] - [(k_1k_2)/(k_{-1} + k_2)]\} = [0.002 \times (8.8/2.3)] = 0.008$, giving an intrinsic CKIE value of 1.066 for the catalyzed route.

It was previously reported that protonated imidazole does not catalyze the decarboxylation of MTh.[6] As a control in the present study, we determined the reaction rate with 1 M imidazole in place of pyridine buffered by 0.3 M acetate (pH 4, $I = 1$, 25 °C). The observed rate constant (6.5×10^{-4} s^{-1}) is equal to that in the presence of acetate alone, which is also a noncatalytic buffer. The unique acceleration by protonated pyridine is consistent with a specific mechanism requiring preassociation. The magnitude of the CKIE for the uncatalyzed reaction (1.058) establishes that C−C bond breaking is a rate-limiting process. The *increase* in the observed CKIE upon addition of pyridine is consistent with a mechanism in which separation of CO_2 and the conjugate base of **2** also limits the overall rate, with protonation by pyridinium enhancing the forward commitment. In the absence of pyridine, the reaction in the reverse direction is more favorable, reducing the net isotope effect from the intrinsic CKIE. This is similar to the situation considered by O'Leary et al.[20] for PEP carboxylase, where an isotope-sensitive step precedes a partially rate-limiting step. In an enzyme, a preassociation mechanism is routinely accessible in an active site, as all components are associated upon binding of the substrate.[17−19]

Interpreting the CKIE of an enzymatic decarboxylation reaction in terms of catalytic commitment[21] is normally based on a comparison with the intrinsic CKIE of the C−C bond-breaking step derived from simple model reactions.[22] Our results suggest that the usual nonenzymatic models may not reveal the intrinsic CKIE by a direct measurement because of the reversibility of the decarboxylation process, which lowers the observed value of the CKIE. To the extent that an enzymatic reaction avoids the readdition of CO_2, the proper intrinsic CKIE for calibration should be that of an irreversible process. Our results show that such a calibration is possible where a catalyst is available that blocks the reverse reaction. We are in the process of investigating the consequences of internal return of carbon dioxide in other reactions.

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