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Isotope Effect, Mechanism, and Origin of Catalysis in the Decarboxylation of Mandelylthiamin

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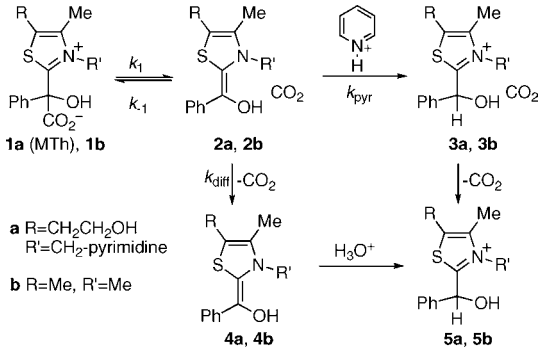
Catalysis is normally understood as resulting from the reduction of activation barriers. Within this idea, the impact of a mechanistic step's catalysis is limited by the degree to which the step is rate limiting and by the size of the barrier before catalysis. A series of recent papers on biomimetic decarboxylations by Kluger and co-workers appears to expand this view of catalysis,[1] as the proposed catalyzed step is the diffusion apart of two neutral simple molecules, normally a nearly barrierless process. By extension, this same phenomenon was suggested to be important in enzymatic catalysis. We find here that the experimental observations in the decarboxylation studied are not consistent with the mechanism and nature of catalysis previously proposed, and we present a more mundane alternative mechanism.

Mandelylthiamin (MTh, **1a**) undergoes decarboxylation in water at pH 5 to 7 with a rate constant of 3×10^{-4} s^{-1}.[1b] This decarboxylation is accelerated, up to a factor of 4, in the presence of pyridinium ions. Notably, *N*-ethylpyridinium ions and neutral protic acids provide no catalysis. To explain these observations, Kluger and co-workers proposed that the decarboxylation step affording the intermediate enol/CO_2 cage **2a** is reversible and "overwhelmingly reverts to the carboxylate" in the absence of catalyst, i.e. $k_{-1} \gg k_{diff}$. Within this proposal, preassociated pyridinium ions would catalyze the reaction by trapping **2a**, preventing reversion to MTh.



This mechanism requires that the reaction of the two adjacent but neutral closed-shell molecules in **2a** be faster than their diffusional separation. However, from known CO_2 diffusion constants[2] and Einsteinian diffusion theory,[3] a free CO_2 molecule in water diffuses on average 5 Å in ~20 ps or 10 Å in ~80 ps. Moreover, the reformation of MTh from **2a** should not be barrierless; M06-2x/6-31+G**/PCM(water)[4] calculations place the enthalpic and free-energy barriers for formation of model **1b** from complex **2b** at 6.3 and 8.5 kcal/mol,[5] leading to a predicted k_{-1} of 4×10^6 s^{-1}. This suggests that reformation of MTh should be on the order of 10 000 times slower than diffusion, precluding the proposed catalytic mechanism.

The experimental ^{13}C kinetic isotope effect (KIE) of 1.058 for the uncatalyzed reaction[1c] of MTh at 25 °C provides more direct evidence against the literature mechanism. We investigated the MTh decarboxylation as well as the mechanism of three contrasting decarboxylations in water (4-pyridylacetic acid,[6,7] trichloroacetate,[8] and 5-nitro-3-carboxybenzisoxazole[9]) by our standard process in which KIE predictions for mechanistic possibilities are compared with the experimental values. Predicted KIEs for fully rate-limiting decarboxylation steps in these reactions were based on transition structures located in M06-2x/PCM calculations. If subsequent diffusional separation of the CO_2 from **2b** (or analogous intermediates for the other molecules) were fully rate limiting, the KIE would be the equilibrium isotope effect for the formation of the intermediate times the KIE for the diffusion step. The necessary equilibrium isotope effects for the four reactions were based on calculated structures and were in a range from 0.989 to 1.003; this corresponds perfectly with known equilibrium ^{13}C isotope effects for typical decarboxylations in water.[10] A KIE for the diffusion step of 1.0007 was assumed based on the experimental effect on the diffusion coefficient for $^{12}CO_2/^{13}CO_2$ in water.[11]

The results are summarized in Table 1. The experimental KIEs do not fit at all with those predicted for rate-limiting diffusion, but the KIE predictions based on fully rate-limiting decarboxylation steps are strikingly accurate. Across the board, these results exclude significant reversibility of the decarboxylation step.

Table 1. Experimental versus M06-2x/6-31+G**/PCM-predicted ^{13}C KIEs ($^{12}k/^{13}k$) for Decarboxylations in Water

system and mechanistic assumption	predicted KIE	experimental KIE
4-Pyridylacetic Acid, 25 °C		
rate limiting decarboxylation	1.054[a,b]	1.057[c]
rate limiting diffusion	0.998	
Trichloroacetate, 70.4 °C		
rate limiting decarboxylation	1.034[b,d]	1.034[e]
rate limiting diffusion	0.990	
5-Nitro-3-carboxybenzisoxazole, 20 °C		
rate limiting decarboxylation	1.049[b]	1.046[f]
rate limiting diffusion	0.998	
MTh, 25 °C		
k_1 fully rate limiting	1.058[b]	1.058[g]
k_{diff} fully rate limiting	1.004	
$k_{-1} \geq 3 \times k_{diff}$	≤1.018	

[a] For a previous KIE prediction, see ref 7. [b] See the Supporting Information for transition structures. [c] See ref 6. [d] Based on the canonical variational transition state at 70.4 °C. [e] See ref 8. [f] See ref 9. [g] See ref 1c.

For the MTh decarboxylation, one must consider an intermediate case in which the decarboxylation step and the subsequent diffusion/pyridinium trapping steps are each partially rate limiting. Assuming propositionally the literature mechanism, the observed rate constant k_{obs} would be governed by eq 1. The right-hand side of eq 1 can never exceed k_1, so the maximum possible acceleration by pyri-

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dinium catalysis, k_{max}/k_{uncat}, is limited by eq 2. No catalysis is possible if $k_{diff} \gg k_{-1}$. The experimentally observed acceleration of at least a factor of 4 would imply that $k_{-1} \geq 3 \times k_{diff}$. This leads to a maximum predicted KIE of 1.018. No realistic combination of alternative assumptions (i.e., among precedented KIEs for decarboxylation or diffusion or reasonable equilibrium isotope effects for formation of **2a**) would lead to a ^{13}C KIE approaching 1.058. In other words, the experimental ^{13}C KIE for the uncatalyzed reaction unambiguously precludes sufficient reversibility in the decarboxylation step to allow any significant catalysis by pyridinium trapping of the intermediate. The catalysis must be explained in another way, and no other evidence discretely implicates reversibility.

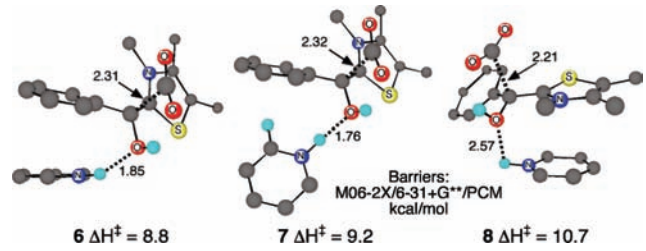
$$k_{obs} = k_1 \frac{k_{diff} + k_{pyr}[PyrH^+]}{k_{-1} + k_{diff} + k_{pyr}[PyrH^+]} \quad (1)$$

$$k_{max}/k_{uncat} = \frac{k_{-1} + k_{diff}}{k_{diff}} \quad (2)$$

The careful work of Kluger and co-workers excluded a number of alternative mechanisms, and one is left to conclude that the pyridinium ions catalyze the reaction by directly affecting the decarboxylation step itself. How? The cation/π interaction of pyridinium ions with arenes is strong in the gas phase,[12] and it remains significant in aqueous solution.[13] In passing from starting MTh to transition state, the phenyl group should become more electron rich and the thiaminium cation evolves into a neutral methylenedihydrothiazol (see the Supporting Information for a discussion of charges in **2**). Both changes favor coordination. We supposed that the pyridinium could coordinate with either the phenyl group or the incipient methylenedihydrothiazol at the transition state. In support of the latter possibility, strong T-shaped and face−face stacked complexes of pyridinium with methylenedihydrothiazol were located, involving interaction energies (MP2/6-311G** + zpe) of 19.7 and 18.1 kcal/mol, respectively.

To explore the potential of a cation/π interaction to catalyze the decarboxylation of MTh, M06-2x/6-31+G**/PCM(water) calculations were employed to locate transition structures for decarboxylation of **1b** complexed with pyridinium. Eighteen such structures were located with pyridinium in various positions and orientations, and eight of these had calculated formal transition state binding enthalpies (defined by the harmonic enthalpy versus that of the uncatalyzed transition structure and separate pyridinium) greater than 6 kcal/mol. The three lowest-enthalpy structures, **6**−**8**, are shown; others are given in the Supporting Information. Structures **6** and **7** were lowest in the M06-2X calculations; structures **6** and **8** were lowest in MP2/6-311+G** single-point energies.

The predicted free-energy barrier for decarboxylation via 6, obtained by including harmonic entropy estimates at a 1 M standard state with the M06-2x/PCM enthalpies, is 1.8 kcal/mol below that of the uncatalyzed reaction. At an experimental pyridinium concentration of 0.4 M, the catalyzed reaction would be predicted to occur about 8 times faster than the uncatalyzed. When the difficulty of the calculation and particularly the simplification of the entropy estimate are considered, this striking agreement with experiment (within 0.4 kcal/mol) is to some degree fortuitous. Nonetheless, the calculated energetics are clearly consistent with an origin of the observed catalysis in pyridinium binding to the transition state.



An intriguing feature of the lowest-energy catalyzed transition structures is that they combine a cation/π face−face or T-shaped interaction with hydrogen bonding to the hydroxyl group. This chelating combination appears critical to the catalysis; the formal transition state binding is unsurprisingly much weaker in the many decarboxylation transition structures exhibiting only one of the interactions. This fits well with the observation that *N*-ethylpyridinium ions and neutral protic acids provide no catalysis. This simple catalysis by transition state binding is also consistent with the observation that the H/D solvent isotope effect on the catalysis is near unity.[14]

In summary, a comparison of predicted and experimental isotope effects shows that there is no significant reversibility in simple decarboxylations in water. From diffusion versus recombination rates, no reversibility is to be expected for the MTh decarboxylation. The calculations suggest that the catalysis that had been the evidence for reversibility arises from simple formal binding to the transition state.

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Supporting Information Available: Complete descriptions of calculations and structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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