Solvent-Accelerated Decarboxylation of *N*-Carboxy-2-imidazolidinone. Implications for Stability of Intermediates in Biotin-Dependent Carboxylations

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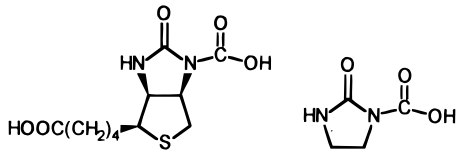
Abstract: The decarboxylation of *N*-carboxy-2-imidazolidinone has previously been established as a model for the transfer of carbon dioxide from *N*(1′)-carboxybiotin. The present paper reports the pH-dependence of the reaction as well as the acceleration of the reaction in methanol and in acetonitrile. These results suggest that enzymic reactions of *N*(1′)-carboxybiotin in a hydrophobic active site with decreased hydrogen bonding can be rapid if the energy of desolvation is compensated by the energy made available by association of the substrate and protein. In addition, a report on the decarboxylation of *N*-carboxy-2-imidazolidinone in organic solvents containing macrocycles (Kluger, R.; Tsao, B. *J. Am. Chem. Soc.* **1993**, *115*, 2089−90) must be reinterpreted on the basis of the inherent instability of the substrate under the reaction conditions.

The transfer of carbon dioxide from the enzymic intermediate *N*(1′)-carboxybiotin to a carbanionic acceptor is a key step in biosynthetic carboxylation pathways.[1] Knowledge of the reactivity patterns of *N*(1′)-carboxybiotin can reveal the potential catalytic functions in an enzymic pathway associated with this intermediate.[2] Caplow and Yager used *N*-carboxy-2-imidazolidinone (**1**) to model the reactivity expected for *N*(1′)-carboxybiotin.[3]



N(1′)-carboxybiotin **1**

They observed that the species is relatively unreactive. If the reactivity of *N*(1′)-carboxybiotin is similar, an enzyme would have to utilize catalytic functions or alter the structure of the cofactor to make it more reactive. Since the formation of *N*(1′)-carboxybiotin on an enzyme may occur in the absence of an acceptor substrate, this stability may be necessary to prevent abortive reactions in which carbon dioxide is transferred to solvent water rather than to a metabolic acceptor. Wallace, Keech, and co-workers showed that in pyruvate carboxylase, a typical biotin-dependent enzyme, the reactivity of *N*(1′)-carboxybiotin is altered once the acceptor substrate (or its analogue) is bound.[4] The mechanism for such activation is unknown although several schemes have been suggested involving structural changes in the protein and cofactor.[2,5,6] An alternative utilization of a structural change in a protein is one that leads to specific stabilization of a transition state relative to a reactant through desolvation.[7,8] The energy for this desolvation can be derived from coupling of favorable binding interactions between the substrate and the enzyme. Could such a mechanism contribute to increased reactivity of *N*(1′)-carboxybiotin upon the binding of substrate? In order to consider this possibility, we have extended the studies of Caplow and Yager[3] to find the effects of the reaction medium on the stability of *N*-carboxy-2-imidazolidinone (**1**).

Experimental Section

General. Chemicals and reagents obtained from commercial suppliers were of the highest available grade. Liquids were distilled. UV spectra were recorded with a double-beam spectrophotometer employing a double-pass monochromator to minimize noise at high absorbance. ^{1}H NMR spectra were recorded at 200 MHz while ^{13}C NMR spectra were recorded at 100 MHz (chemical shifts for carbon refer to dioxane at 67.4 ppm). pH measurements were done with a meter equipped with a combination electrode. IR spectra were performed on a FT instrument. Kinetic data were fit to integrated rate expressions by nonlinear regression on a computer.

Synthesis. *N*-carbomethoxycarbonyl-2-imidazolidinone was prepared according to the published procedure.[9,10] The product was crystallized from water: mp 173−175 °C; ^{1}H NMR ($CDCl_3$) δ 3.51 and 3.93 (m, 4H, $-CH_2CH_2-$), 3.86 (s, 3H, $-OCH_3$), 6.45 (broad, 1H, NH); ^{13}C NMR ($CDCL_3$) δ 36.84 and 43.31 (N$-CH_2CH_2-$N), 53.46 ($-OCH_3$), 152.50 (N$-$CO$-$O), 156.31 (N$-$CO$-$N); IR (KBr) 1669, 1761 cm^{-1}.

N-Carboxy-2-imidazolidinone (**1**) was obtained by hydrolysis of *N*-methoxycarbonyl-2-imidazolidinone.[3] The ester (0.13 g, 0.9 mmol) was suspended in 10 mL of water. Potassium hydroxide solution (1.8 mL, 1 M) was added with stirring. After 15 min, the solution was freeze-dried. NMR analysis revealed that the residue is an 80:20 mixture of *N*-carboxy-2-imidazolidinone and 2-imidazolidinone. Since decarboxylation of *N*-carboxy-2-imidazolidinone is slow under these conditions, the mixture results from the carbamate ester undergoing both C−N and C−O cleavage. Other reaction conditions gave lower proportions of the desired product. All conditions attempted for separation led to decomposition of the desired material. For **1**: ^{1}H NMR (D_2O) δ 3.38 and 3.73 (two multiplets, 4H); ^{13}C NMR (D_2O)

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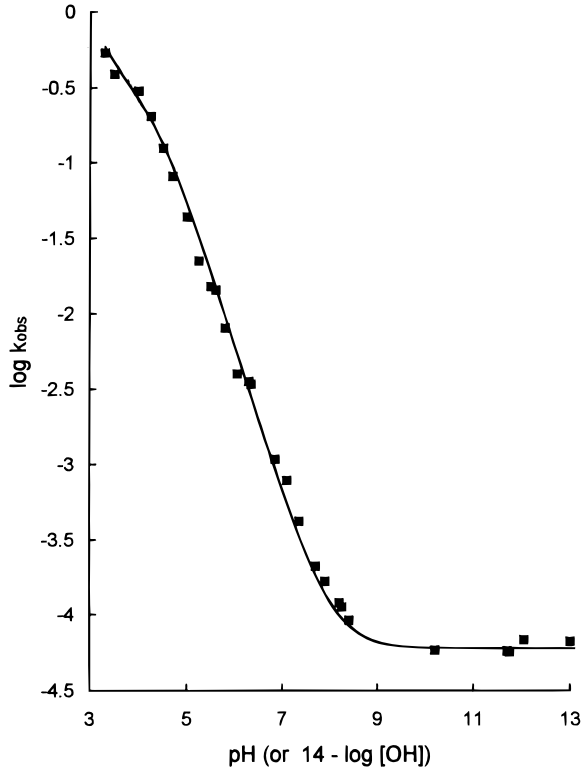


Figure 1. Log of observed rate constant for decarboxylation of **1** as a function of solution acidity (water, 25 °C; see Experimental Section).

36.99 and 45.06 (N−CH_2CH_2−N), 158.25 (N−CO_2^-), 161.38 (N−CO−N); IR (KBr) 1661, 1723 cm^{-1}.

Kinetics. Solutions of *N*-carboxy-2-imidazolidinone (0.005 g in 0.2 mL of water) were stored on ice and kept for no longer than 8 h. Samples contained about 20% of the decarboxylation product, 2-imidazolidinone (from the initial preparation). Since the decarboxylation reaction is first order in substrate and is irreversible, the presence of the product does not affect the results. Reactions were maintained at 25.0 °C with a circulating bath. Portions of the solution (0.005 mL) were added by microsyringe to 3 mL of 0.010 M buffer (ionic strength maintained at 0.1 with potassium chloride). The decarboxylation of **1** was monitored by observing the decrease in absorbance at 200 nm. The double-pass monochromator instrument was necessary for these measurements due to high background absorbance at this frequency. Acetate buffers were used at pH 3.5−5.2, MES at pH 5.5−6.8, HEPES at pH 7.0−8.0, BICINE at pH 8.2−9.0, and phosphate at pH 10−12. The data were fit to the integrated first-order rate equation by nonlinear regression.

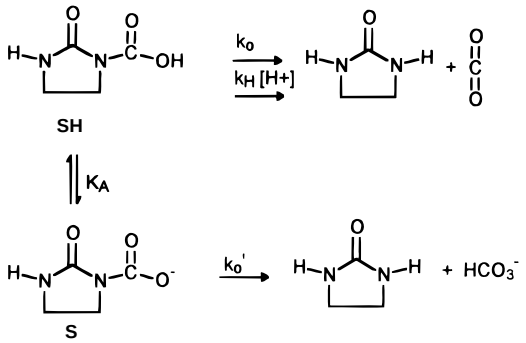
Kinetics utilizing ^{1}H NMR were conducted at the temperature of the instrument's probe (22 °C) in 0.6 mL of phosphate buffer (0.5 M in D_2O, pD 9.0). Spectra were recorded at intervals, and relative concentrations were estimated from the integrals and normalized against the residual HDO peak at δ 4.85. The reaction was followed by measuring the integrated intensity of the signals from the product at δ 3.38 and 3.82. This was accompanied by the disappearance of the signal of the reactant of δ 3.50.

Rate data for the reaction in other solvents were collected under the conditions described for aqueous reactions monitored with the UV spectrophotometer. Methanol and acetonitrile in aqueous mixtures were used for observation of solvent effects. Reactions were initiated by adding a stock solution containing the substrate (typically 0.005 mL) to 3 mL of the solvent mixture. The reaction product was identified as imidazolidinone by isolation of samples run on a preparative scale, followed by ^{1}H NMR analysis.

Results

The observed first-order rate coefficients for decarboxylation of *N*-carboxy-2-imidazolidinone as a function of pH are shown in Figure 1. The curve contains a concave upward turn. This curve requires the existence of two mechanisms for the conversion, with each dominant in a different range of acidities. On the basis of the structure of **1**, it is reasonable to expect that these mechanisms are (1) reaction of the neutral, undissociated form of **1** (shown as "SH" in Scheme 1) leading to a transition state with no net change in protonation and (2) reaction via a transition state at the same state of protonation as the conjugate base of **1** ("S" in Scheme 1).

Scheme 1



Thus, the rate data were fit to the expression (points at high acidity show a small upward deviation that may indicate acid catalysis but the term is too small to evaluate with certainty):

$$v = k_{obs}[S]_T = k[SH] + k'[S] \quad (1)$$

The observed first-order rate coefficient is k_{obs}, and $[S]_T$ is the concentration of *N*-carboxy-2-imidazolidinone in the reaction solution. The values for the rate constants that give a best fit to the data were sued to generate the plot relating k_{obs} to pH. Scheme 1 summarizes the kinetic terms and formal mechanisms.

The relationship between k_{obs} and the specific rate constants in eq 1 was obtained by substituting the value of the acid dissociation constant of *N*-carboxy-2-imidazolidinone and the conservation expression.

$$[S]_T = [SH] + [S] \quad (2)$$

$$K_A = [S][H^+]/[SH] \quad (3)$$

$$k_{obs} = (k[H^+] + k'K_A)/(H^+ + K_A) \quad (4)$$

At pH > 8, the rate of reaction is independent of pH. This is consistent with a mechanism in which carbon dioxide is lost from the ionized form of the substrate S. The simplified rate expression for the observed rate coefficient under these conditions is $k_{obs} = k' = 5.2 \times 10^{-5}$ s^{-1}. Below pH 8, the reaction increases in rate with increasing acidity. At pH < 8, the rate is proportional to acid concentration ($k_{obs} = k[H^+]/K_a$), requiring reaction via a neutral transition state. The acidity constant K_a (5.6×10^{-5}, $pK_a = 4.2$) and k ($= 0.35$ s^{-1}) were obtained from a self-consistent fit of the data to eq 4.

Studies of solvent effects on reactions were conducted in combinations of organic solvents and aqueous solutions at pH = 10.2. At this acidity there is no net protonic change from the reaction: the protonation of the conjugate base of the imidazolidinone product is compensated by the release of a proton that results from formation of carbonic acid. In a control experiment, the pH of the reaction in water initially at pH 10.2 was monitored for 10 half-lives. The acidity remained constant throughout the reaction with first-order kinetics as described in Table 1. The rate of the reaction increases as increasing amounts of methanol replace water. In pure methanol, the rate

Table 1. Solvent Effects on the Decarboxylation of **1** at 25 °C (samples prepared at pH 10.2)

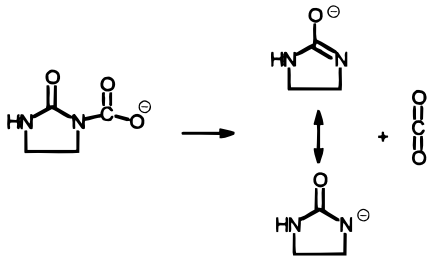
solvent	10^5k (s^{-1})	k_{rel}
water (pH 10.2)	5.2	1
33.3% MeOH	10.3	1.8
66.6% MeOH	33.7	6
100% MeOH	273.0	48.7
33.3% CH_3CN	12.1	2.2
66.6% CH_3CN	39.0	7.0
100% CH_3CN	2680	517

Table 2. Temperature Dependence of the Rate Coefficients for Decarboxylation of **1**

pH	T (°C)	k (s^{-1})
6.1[a]	25.0	4.0×10^{-3}
	35.0	1.2×10^{-2}
	45.0	3.2×10^{-2}
10.2[b]	25.0	6.1×10^{-5}
	45.0	6.5×10^{-4}
	65.0	4.4×10^{-3}

[a] MES buffer (10 mM).

Scheme 2



is 50 times that in pure water (Table 1). The acceleration is an order of magnitude greater in acetonitrile (520 times).

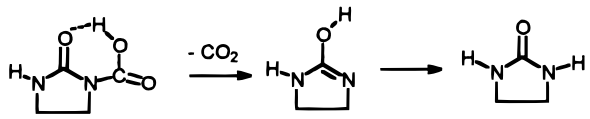
Activation parameters for the reaction in water are the following: $\Delta H^{\ddagger} = 20$ kcal/mol and $\Delta S^{\ddagger} = 56$ eu, pH 10.2; $\Delta H^{\ddagger} = 22$ kcal/mol and $\Delta S^{\ddagger} = 54$ eu, pH 6.1, calculated from the data in Table 2.

Discussion

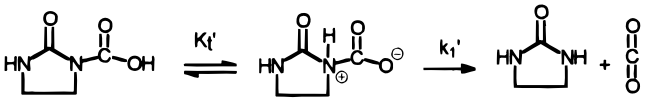
N-Carboxy-2-imidazolidinone is stable in neutral aqueous solution, consistent with earlier reports.[3] The predominant form is the anion, with $t_{1/2} = 3.2$ h for decarboxylation at 25 °C. The undissociated form of *N*-carboxy-2-imidazolidinone is much more reactive, with a first-order rate constant for decarboxylation 6000 times greater than that of the anion. This large difference suggests that the proton makes available a route that significantly stabilizes the transition state. For the anion, the reaction should follow the reaction patterns of carbonate monoesters[11] where the unimolecular transition state produces carbon dioxide concerted with departure of the leaving group, in this case the cyclic urea enolate (Scheme 2).

The mechanism of reaction of the neutral species must be consistent with its much larger reaction rate. One possibility is a parallel to the family of mechanisms for the decarboxylation of 3-ketocarboxylic acids in which the equivalent of the proton of the carboxyl group becomes associated with the β-carbonyl group in the transition state.[12,13] In this mechanism,

Scheme 3



Scheme 4



the presence of an internal hydrogen bond provides stabilization that is not on the path to the transition state for the decarboxylation (Scheme 3).

Alternatively, formation of one of the N-protonated zwitterionic tautomers could lead directly to the keto form, avoiding the high-energy enol. Protonation of the carbamate nitrogen also destroys π overlap across the C−N bond. If this species is sufficiently reactive it may represent a transition state rather than an intermediate (Scheme 4).

The rate-determining transition state involves fragmentation in either process and is consistent with the large and positive $\Delta S^{\ddagger} = 65$ eu. (The enthalpy of activation $\Delta H^{\ddagger} = 20$ kcal/mol is normal for decarboxylation reactions in which the developing stability of carbon dioxide contributes to the reduced potential energy of the transition state.)[14]

The decarboxylation of *N*-carboxy-2-imidazolidinone is more rapid in solvents less polar than water, paralleling the trend in dielectric constant of the medium (Table 1; the dielectric constants are water (78.3), acetonitrile (37.5), and methanol (32.6).[15] Acceleration in organic solvents necessarily results from more favorable solvation of the transition state or less favorable solvation of the reactant or both.[16] The observed rates show an inverse correlation to the hydrogen-bonding ability of the solvent, suggesting that decreasing such interactions may contribute to the acceleration.

The possibility of more favorable solvation of the transition state for decarboxylation is difficult to visualize from simple models if the neutral form of *N*-carboxy-2-imidazolidinone reacts in the less polar solvents. However, if the increase in rate is due to the conjugate base becoming much more reactive, then the transition state may contain a more delocalized charge that can be better solvated in lower dielectric media.

The subject of accelerated decarboxylation in nonaqueous media has focused on 3-carboxybenzoxyisoxazoles. The reactions are accelerated to very large extents in solvents of lower polarity and decreased hydrogen-binding ability.[17,18] Theoretical studies of this reaction have provided detailed insights into the factors controlling the reaction.[19,20] Hilvert and co-workers relate the catalytic properties of antibodies in the decarboxylation reaction to solvent disruption of hydrogen bonds between ion pairs.[21,22] For *N*-carboxy-2-imidazolidinone, H-bonding should

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stabilize the reactant but not the transition state. Thus, we see a greater acceleration in acetonitrile than in methanol.

Since these results establish the instability of *N*-carboxy-2-imidazolidinone in nonaqueous solutions, we must reconsider aspects of an earlier report from our laboratory.[23] It was reported that the addition of a macrocyclic compound that selectively binds derivatives of *N*-carboxy-2-imidazolidinone enhances the rate of decarboxylation of *N*-carboxy-2-imidazolidinone. However, the kinetic measurements were carried out on samples in organic solvents where we now know that the lifetime of the substrate is less than the time of the first measured point. A change in absorbance with time was noted, and the final product is 2-imidazolidinone. This means that the product did not come from the presumed substrate. The result can be explained by reaction of the ester which is the synthetic precursor of *N*-carboxy-2-imidazolidinone. (We observed the presence of this impurity in a sample that had been retained from the earlier synthesis.) The small amount of water in the reaction solution facilitates hydrolysis, which is likely to be the kinetic process we observed, since once hydrolysis is complete, decarboxylation is rapid. Thus, conclusions that those results implicate potential enzymic modes of activation are incorrect.

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Conclusions

The instability of *N*-carboxy-2-imidazolidinone in nonaqueous solutions compared to that in water can be extrapolated to a mode of enzymic activation of *N*(1′)-carboxybiotin. The substrate-induced activation of *N*(1′)-carboxybiotin is a subject that has led to a number of proposals of mechanisms and pathways but desolvation has not been considered.[2,6,24,25] If the energy for the transfer of enzyme-bound *N*-carboxybiotin into a hydrophobic region is made possible by binding of the carboxylation acceptor, then a good deal of the stability of *N*(1′)-carboxylation is readily overcome. Structural information on the environment of such a site can be analyzed to consider the reasonableness of such a possibility.[26]

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