Communications to the Editor

The Rate of Spontaneous Decarboxylation of Amino Acids

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Amino acids are far more stable than the bonds that join them in polypeptides,[1] remaining intact for long periods at high temperatures in the solid phase[2,3] and in acid solution.[3] Their principal route of biological decomposition involves the catalytic action of decarboxylation enzymes that use enzyme-linked pyridoxal phosphate or pyruvoyl groups as cofactors.[4] Several amino acid decarboxylases have attracted interest as potential targets for inhibitor design, because of their involvement in generating hormones and neurotransmitters such as epinephrine, serotonin, and γ-aminobutyric acid.[5] To appreciate the effectiveness of these enzymes as catalysts, it would be desirable to have quantitative information about the susceptibility of amino acids to spontaneous decarboxylation in neutral solution. Here, we show that the decarboxylation of amino acids proceeds even more slowly than the decarboxylation of orotidine 5′-phosphate, the slowest biological reaction whose spontaneous rate has been reported in the absence of an enzyme.[6]

Glycine (0.05 M) in potassium phosphate buffer (0.1 M, pH 6.8) was heated for various time intervals in quartz tubes that had been sealed under vacuum to remove oxygen, over the temperature range between 170 and 260 °C. After the tubes had cooled, the concentrations of glycine and methylamine were determined by comparing the integrated intensities of their proton magnetic resonances in D_2O to which pyrazine had been added as an integration standard. The spontaneous decarboxylation of glycine, present almost entirely as the zwitterion at pH 6.8, to yield methylamine was found to proceed to completion following first-order kinetics. The rate of decarboxylation of glycine did not vary significantly in phosphate buffers in the pH range (measured at room temperature) between pH 5.8 to 7.8. However, decarboxylation proceeded approximately 10 times more slowly in solutions in which 0.91 equiv of HCl or 0.91 equiv of KOH had been mixed with the zwitterionic amino acid. Thus, the anionic and cationic forms of glycine appear to be at least 10-fold less reactive than the zwitterion. Decarboxylation was found to be retarded by increasing ionic strength (KCl), as expected for a reaction involving charge dispersal in the transition state (data not shown). Methyl substitution on glycine produced only a modest effect on the rate of decarboxylation of glycine: thus, rate constants observed for glycine, alanine, sarcosine, and *N*,*N*-dimethylglycine were 3.6×10^{-6}, 4.7×10^{-6}, 17×10^{-6}, and 2.0×10^{-6} s^{-1}, respectively, at 200 °C.

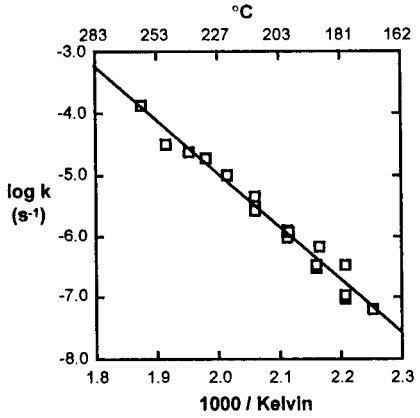

Figure 1. Arrhenius plot of first-order rate constants for decarboxylation of glycine to methylamine, in potassium phosphate buffer (0.1 M, pH 6.8), over the temperature range from 170 to 260 °C.

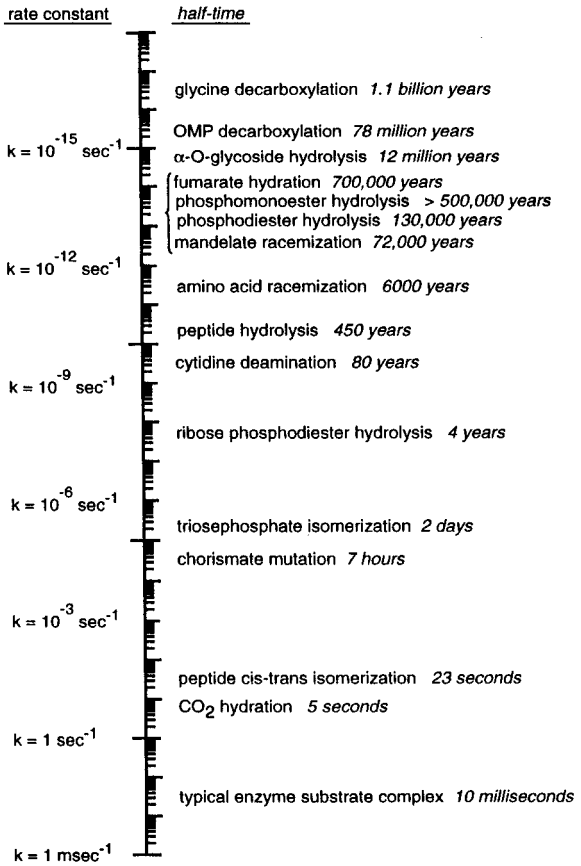

Figure 2. Half-times of biological reactions proceeding spontaneously at 25 °C in neutral solution in the absence of a catalyst.[7]

Attempting to observe the decarboxylation of betaine, we found that the trimethylammonium group of this glycine analogue undergoes hydrolysis to yield dimethylglycine and methanol too rapidly to allow the rate of decarboxylation of the starting material to be established.

First-order rate constants for glycine decarboxylation obtained in potassium phosphate buffer (0.1 M, pH 6.8), over the temperature range from 170 to 260 °C, yielded a linear Arrhenius

(1) (a) Bryant, R. A. R.; Hansen, D. E. *J. Am. Chem. Soc.* **1996**, *118*, 5498−5499. (b) Radzicka, A.; Wolfenden, R. *J. Am. Chem. Soc.* **1996**, *118*, 6105−6109.
(2) Abelson, P. H. *Sci. Am.* **1956**, *195*, 83−91.
(3) Conway, D.; Libby, W. F. *J. Am. Chem. Soc.* **1958**, *80*, 1077−1080.
(4) Boeker, E. A.; Snell, E. E. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1972; Vol. 6, pp 217−254.
(5) Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: New York, 1992; Chapter 5, pp 188−197.
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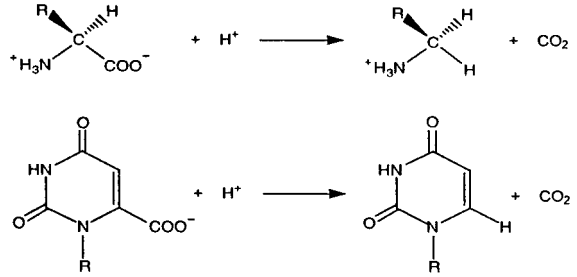
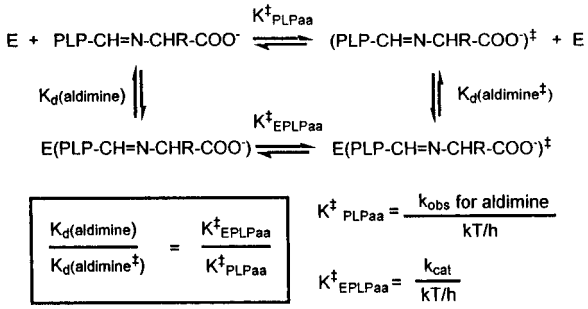
Scheme 1. Decarboxylation of an Amino Acid (R = H for glycine) and of Orotidine 5′-phosphate (R = 5-phosphoribosyl-).



plot (Figure 1) that could be extrapolated to yield a rate constant of $\sim 2 \times 10^{-17}$ s^{-1} at 25 °C, with an enthalpy of activation of 39 (± 2) kcal/mol. The half-time for this reaction at 25 °C in neutral solution is 1.1 billion years, somewhat longer than the half-time (78 million years) reported earlier for the spontaneous decarboxylation of orotic acid derivatives in neutral solution (Figure 2). Orotidine 5′-phosphate can be considered a modified amino acid (Scheme 1), with its 1-nitrogen atom joined in glycosidic linkage to ribose 5-phosphate. Despite the slow progress of these reactions, they proceed much more rapidly than the decarboxylation of glycolic or acetic acids, consistent with the electron-withdrawing influence of an α-nitrogen atom. In neutral phosphate buffer, glycolate was decarboxylated to methanol only at temperatures above 300 °C, and acetate had undergone no detectable decarboxylation after 2 weeks at 360 °C.

If the value of k_{cat} for the pyridoxal-dependent arginine decarboxylase of *Escherichia coli* (1375 s^{-1})[8] is compared with the present rate constants for spontaneous decarboxylation of glycine and related amino acids under the same conditions (2×10^{-17} s^{-1}), arginine decarboxylase is seen to enhance the rate of reaction by a factor (k_{cat}/k_{non}) of 7×10^{19}. This rate enhancement somewhat exceeds the rate enhancement ($k_{cat}/k_{non} = 1.4 \times 10^{17}$) reported earlier for orotidine 5′-phosphate decarboxylase (ODCase).[6] Although these reactions are of comparable difficulty, it should be noted that ODCase differs from amino acid decarboxylases in that it acts as a pure protein catalyst, without the intervention of metals or other cofactors.

Scheme 2. Relative Enzyme Binding Affinities for an Aldimine in the Ground State and Transition State for Decarboxylation.



Because amino acid decarboxylases depend on cofactors (pyridoxal or pyruvate[9]) that react covalently with the substrate to form an aldimine intermediate, there is a fundamental difference in mechanism between the enzymatic and the uncatalyzed reaction, and the term "transition state affinity" loses it conventional meaning.[10] It remains possible, however, to compare the catalytic effectiveness of such an enzyme with that of the cofactor alone. Zabinski and Toney (personal communication) have recently demonstrated that in the absence of enzyme, PLP catalyzes the decarboxylation of α-aminoisobutyric acid, with a turnover number of 3.9×10^{-6}s^{-1} for the aldimine at pH 5. Comparison of this value with the present rate constant indicates that PLP alone enhances the rate of decarboxylation by a factor of 2×10^{11}. As noted in Scheme 2, the much greater rate enhancement produced by arginine decarboxylase ($k_{cat}/k_{non} = 7 \times 10^{19}$) provides a measure of this protein's contribution to catalysis. The difference between these factors (3.5×10^{8}-fold) can be considered to set a lower limit on the factor by which the protein binds the aldimine more tightly in the transition state than in the ground state for decarboxylation.

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(7) (a) Glycine decarboxylation (this work). (b) α-*O*-glucoside hydrolysis (Wolfenden, R.; Lu, S.; Young, G. *J. Am. Chem. Soc.* **1998**, *120*, 6814−6815). (c) Fumarate hydration (Bearne, S. L.; Wolfenden, R. *J. Am. Chem. Soc.* **1995**, *117*, 6588−6589). (d) Phosphomonoester and phosphodiester hydrolysis (Wolfenden, R.; Ridgeway, C.; Young, G. *J. Am. Chem. Soc.* **1998**, *120*, 833−834). (e) Mandelate racemization (Bearne, S. L.; Wolfenden, R. *Biochemistry* **1997**, *36*, 1646−1656). (f) Amino acid racemization (Bada, J. L. *J. Am. Chem. Soc.* **1992**, *94*, 1371−1373). (g) Peptide hydrolysis (ref 1b). (h) Cytidine deamination (Snider, M. J.; Gaunitz, S.; Ridgway, C.; Short, S. A.; Wolfenden, R. *Biochemistry* **2000**, *39*, 9746−9753). (i) Ribose phosphodiester hydrolysis (Thompson, J. E.; Kutateladze, T. G.; Schuster, M. C.; Venegas, F. D.; Messmore, J. M.; Raines, R. T. *Bioorg. Chem.* **1995**, *23*, 471−481); and other reactions (ref 6).

(8) Blethen, S. L.; Boeker, E. A.; Snell, E. E. *J. Biol. Chem.* **1968**, *243*, 1671−1677.

(9) The decarboxylation of amino acids is the slowest biological process whose rate appears to have been reported in the absence of a catalyst. When one considers how such a process might have originated, pyruvic acid seems more likely than pyridoxal phosphate to have arisen spontaneously as a cofactor for amino acid decarboxylation under conditions prevailing on the primitive earth. It may be worth noting that small quantities of pyruvate esters have been shown to be generated from carbon monoxide and formic acid at high temperature and pressure in a reducing environment (Cody, G. D.; Boctor, N. Z.; Filley, T. R.; Hazen, R. M.; Scott, J. H.; Sharma, A.; Yoder, H. S., Jr. *Science* **2000**, *289*, 1337−1340).

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