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H/D Kinetic Isotope Effect as a Tool to Elucidate the Reaction Mechanism of Methyl Radicals with Glycine in Aqueous Solutions

Guy Yardeni,*[†,‡] Israel Zilbermann,[†,‡] Eric Maimon,[†,‡] Lioubov Kats,[‡] Ronen Bar-Ziv,[†,‡] and Dan Meyerstein*[‡,§]
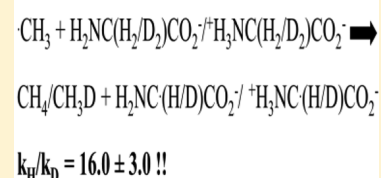
[†]Chemistry Department, Nuclear Research Centre Negev, Beer-Sheva, 86000, Israel
[‡]Chemistry Department, Ben-Gurion University of the Negev, Beer-Sheva, 84105, Israel
[§]Biological Chemistry Department, Ariel University, Ariel, 44837, Israel

ABSTRACT: The H/D kinetic isotope effect (KIE) for the reaction of methyl radicals with glycine in aqueous solutions at pH 10.6 equals 16 ± 3. This result proves that the methyl radical abstracts a hydrogen atom from the methylene group of glycine and not an electron from the unpaired couple on the nitrogen atom. The rate constant of the reaction of methyl radicals with glycine at pH 7.0 is orders of magnitude smaller than that at pH 10.6.

Large Kinetic Isotope Effect:

$$^{\bullet}CH_3 + H_2NC(H_2/D_2)CO_2^-/^+H_3NC(H_2/D_2)CO_2^- \rightarrow$$

$$CH_4/CH_3D + H_2NC^{\bullet}(H/D)CO_2^-/^+H_3NC^{\bullet}(H/D)CO_2^-$$

$$k_H/k_D = 16.0 \pm 3.0 \text{ !!}$$

INTRODUCTION

The reactions of alkyl radicals and alkyl-peroxyl radicals with organic substrates are key steps in a variety of catalytic[1−4] and physiological processes.[5,6] The latter are usually initiated by hydroxyl radicals,[7] which react in very fast reactions with most components of biological systems.[8] The rate constants of the reactions of hydroxyl radicals with ammines and amino acids are pH-dependent, the rate constants for RNH_2 being considerably higher than those for RNH_3^+.[9] This increase is usually attributed to the delocalization, reorganization energy of the radical $H_2NCR^1R^{2\bullet}/H_2NCRCO_2^{\bullet-}$.[10,11] However it might also be due to an electron abstraction from the lone pair on the nitrogen of RNH_2 followed by an α H atom shift forming the same radical. Recent results point out that the reaction of hydroxyl radicals with glycine involves three very short-lived intermediates:[12] (a) $H_2NCH_2CO_2^-{}^{\bullet\bullet\bullet}HO^{\bullet}$ that decomposes to yield the radicals $H_2NC^{\bullet}HCO_2^-$ and $^{\bullet}NHCH_2CO_2^-$; (b) $HO^{\bullet}.{}^{\bullet}NH_2CH_2CO_2^-$ or $HO^-{}^{\bullet\bullet\bullet} + {}^{\bullet}NH_2CH_2CO_2^-$ that decomposes to yield the radicals $NH_2CH_2^{\bullet}$ and $^{\bullet}NHCH_2CO_2^-$.[12] It seemed therefore of interest to measure the kinetics and mechanism of the simplest alkyl radical, methyl, with the simplest amino acid, glycine, in order of differentiating between the plausible mechanisms. It should be noted that $^{\bullet}CH_3$ radicals differ from $^{\bullet}OH$ radicals in two aspects: (a) They are weaker oxidizing agents as the C−H formed in their reactions is weaker than the O−H bond. (b) $^{\bullet}CH_3$ radicals are hydrophobic and are not expected to form transients with hydrogen bonds, e.g., $H_2NCH_2CO_2^-{}^{\bullet\bullet\bullet}HO^{\bullet}$. It was decided to measure the kinetic isotope effect (KIE) of the process, as it is well established that H atom abstraction from C−H groups by radicals involves a large KIE.[13,14] In 1970, Moger et al. estimated that the rate constant of this reaction at pH 11.5 is 120 M^{-1} sec^{-1} and that the KIE = 10.5.[15] These were clearly rough estimates as the results presented herein point out.

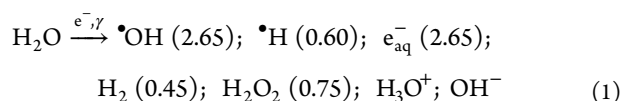## MATERIALS AND METHODS

Materials. All solutions were prepared from analytical grade chemicals. Glycine and D_2-glycine, $H_2NCD_2CO_2H$, were purchased from Sigma-Aldrich. Dimethyl sulfoxide was purchased from Bio-Lab. The water used was heat distilled and then passed through a Millipore setup, the final resistance being above 15 $M\Omega$ cm^{-1}. Solutions were deaerated by $N_2O_{(g)}$ purchased from "Maxima". pH values were measured with a Hanna HI 9321 pH meter and adjusted with $HClO_4$ and/or NaOH solutions.

Irradiations. γ-Irradiations were carried out in a ^{60}Co γ source, G-220 Gammacell, with a dose rate of 13 $Gy\,min^{-1}$, which was determined by means of Fricke dosimetry using a G value of $G = 15.6$.[16,17]

Gas Chromatographic Analysis. GC analyses were performed using a HP 5890 Series II gas chromatograph with a pack Q column and a flame ionization detector (FID).

Production of Radicals. $^{\bullet}CH_3$ radicals were prepared by irradiating N_2O-saturated aqueous solutions containing $(CH_3)_2SO$ via the following reactions: The primary radicals formed when dilute aqueous solutions are irradiated with ionizing radiation are

$$H_2O \xrightarrow{e^-,\gamma} {}^{\bullet}OH\ (2.65);\ {}^{\bullet}H\ (0.60);\ e_{aq}^-\ (2.65);$$

$$H_2\ (0.45);\ H_2O_2\ (0.75);\ H_3O^+;\ OH^- \qquad (1)$$

In parentheses are given the relative yields of the primary products, expressed as G values.[18] (G values are defined as the number of species of each product formed per 100 eV of

Table 1. Yield of Products and Rate Constants for the Reaction of Methyl Radicals with Glycine and Deuterated Glycine[a]

solution composition	$G(CH_4)$	$G(C_2H_6)$	$G(total) = G(CH_4) + 2G(C_2H_6)$	$G(CH_4)/G(C_2H_6)$	k_7 (M^{-1} sec^{-1})
0.10 M $(CH_3)_2SO$, N_2O sat., pH 7.0	2.35	1.86	6.06	1.27	
0.0010 M glycine, 0.10 M $(CH_3)_2SO$, N_2O sat., pH 7.0	2.50	1.95	6.39	1.28	$k < 3 \times 10^2$
0.10 M $(CH_3)_2SO$, N_2O sat., pH 10.6	2.39	2.04	6.47	1.18	
0.0010 M glycine, 0.10 M $(CH_3)_2SO$, N_2O sat., pH 10.6	3.89	1.02	5.92	3.82	$(2.0 \pm 0.6) \times 10^4$
0.0010 M D_2-glycine, 0.10 M $(CH_3)_2SO$, N_2O sat., pH 10.6	2.82	1.83	6.47	1.55	$(1.3 \pm 0.4) \times 10^3$
0.020 M $(CH_3)_2SO$, N_2O sat., pH 10.6	0.99	2.98	6.95	0.33	
0.0010 M glycine, 0.020 M $(CH_3)_2SO$, N_2O sat., pH 10.6	2.87	0.98	4.82	2.95	$(2.5 \pm 0.8) \times 10^4$
0.0010 M D_2-glycine, 0.020 M $(CH_3)_2SO$, N_2O sat., pH 10.6	1.39	2.49	6.36	0.56	$(1.5 \pm 0.4) \times 10^3$

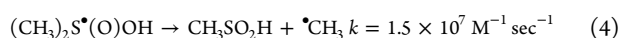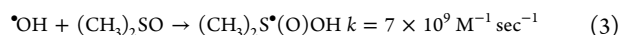[a]All the solutions are N_2O-saturated and were irradiated to 200 Gy.

radiation absorbed by the solution). The distribution of these products in the solution after 1×10^{-7} s is homogeneous.[18]

In N_2O saturated solutions, $[N_2O] = 2.2 \times 10^{-2}$ M,[19] are irradiated, the hydrated electrons are transformed into $^\bullet$OH radicals via[20]

$$e^-_{aq} + N_2O \xrightarrow{H_2O} N_2 + OH^- + {}^\bullet OH \quad k = 9.1 \times 10^9 \text{ M}^{-1}\text{ sec}^{-1} \quad (2)$$

Thus, at pH > 3, $G(^\bullet OH) = 6.0$ is obtained.[20]

In the presence of $(CH_3)_2SO$ the $^\bullet$OH radicals are converted into $^\bullet CH_3$ radicals via[21]

$${}^\bullet OH + (CH_3)_2SO \rightarrow (CH_3)_2S^\bullet(O)OH \quad k = 7 \times 10^9 \text{ M}^{-1}\text{ sec}^{-1} \quad (3)$$

$$(CH_3)_2S^\bullet(O)OH \rightarrow CH_3SO_2H + {}^\bullet CH_3 \quad k = 1.5 \times 10^7 \text{ M}^{-1}\text{ sec}^{-1} \quad (4)$$

In the absence of other solutes the $^\bullet CH_3$ radicals thus formed are transformed into CH_4 and C_2H_6 via[22,23]

$${}^\bullet CH_3 + (CH_3)_2SO \rightarrow CH_4 + {}^\bullet CH_2(CH_3)SO \quad k = 100 \pm 20 \text{ M}^{-1}\text{ sec}^{-1} \quad (5)$$

$${}^\bullet CH_3 + {}^\bullet CH_3 \rightarrow C_2H_6 \quad k = 1.6 \times 10^9 \text{ M}^{-1}\text{ sec}^{-1} \quad (6)$$
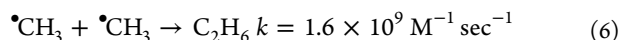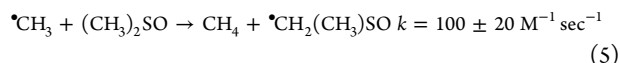
The relative yields of CH_4 and C_2H_6 thus depend on the concentration of $(CH_3)_2SO$ and the dose rate of irradiation.

RESULTS

Aqueous solutions of glycine and D_2-glycine were irradiated in the presence of $(CH_3)_2SO$ in the ^{60}Co γ source, and the yields of the final products (methane and ethane) were measured; the results are summed up in Table 1.

The rate constant of the reaction of the methyl radical with glycine and D_2-glycine were calculated using the assumption that all the ethane is formed via reaction 6:

$$(d[C_2H_6]/dt) = k_6[^\bullet CH_3]_{ss}^2 \quad (I)$$

Where $[^\bullet CH_3]_{ss}$ is the steady-state concentration of $^\bullet CH_3$ during the irradiation.

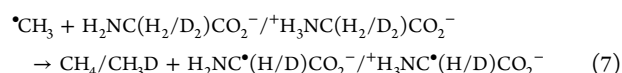$$(d[CH_4]/dt) = k_5[^\bullet CH_3]_{ss}[(CH_3)_2SO] + k_7[^\bullet CH_3]_{ss}[(glycine)] \quad (II)$$

At steady state, i.e., during the irradiation, $d[^\bullet CH_3]_{ss}/dt = 0$. Therefore

$$0 = d[^\bullet CH_3]_{ss}/dt = ((\text{dose rate, Gy min}^{-1})G(^\bullet CH_3) \times 10^{-7}/60)$$
$$- 2k[^\bullet CH_3]_{ss}^2 - k_5[^\bullet CH_3]_{ss}[(CH_3)_2SO] - k_7[^\bullet CH_3]_{ss}(glycine) \quad (III)$$

The dose rate of the ^{60}Co γ source was 13 Gy min^{-1} and $G(^\bullet CH_3) = 6$.[21]

$$[C_2H_6]/[CH_4] = k_6[^\bullet CH_3]_{ss}^2/(k_5[^\bullet CH_3]_{ss}[(CH_3)_2SO]$$
$$+ k_7[^\bullet CH_3]_{ss}[(glycine)])$$
$$= k_6[^\bullet CH_3]_{ss}/(k_5[(CH_3)_2SO] + k_7[(glycine)]) \quad (IV)$$

As k_5, k_6, and all the concentrations are known, measuring the yields of C_2H_6 and CH_4 enables the calculation of k_7.[24] (The pK_a of glycine is 9.6.)[24]

$${}^\bullet CH_3 + H_2NC(H_2/D_2)CO_2^-/{}^+H_3NC(H_2/D_2)CO_2^-$$
$$\rightarrow CH_4/CH_3D + H_2NC^\bullet(H/D)CO_2^-/{}^+H_3NC^\bullet(H/D)CO_2^- \quad (7)$$

The results clearly point out that the rate constants of reaction 7 at pH 10.6 are several orders of magnitude higher than those at pH 7.0. This result is in accord with an earlier report;[15] however, the rate constants determined in this study are 2 orders of magnitude higher than those previously suggested.[15] The redox potential for $(NH_2CHCOO^{\bullet-}/NH_2CH_2COO^-)$ is 0.8 V, and the redox potential for $(^+NH_3CHCOO^{\bullet-}/^+NH_3CH_2COO^-)$ is 1.5 V,[25] explaining the pH effect on k_7. The observation that k_7 for $H_2NC(H_2/D_2)CO_2^-$ is orders of magnitude higher than that for $^+H_3NC(H_2/D_2)CO_2^-$ is in accord with the analogous reaction of $^\bullet$OH radicals with glycine and other amino acids and amines.[26,27] This points out the effect of the delocalization, reorganization energy of the radical $H_2NC(H/D)CO_2^{\bullet-}$ and the effect of the $-NH_3^+/NH_2$ groups on the bond dissociation energy of $^+H_3NC(H_2/D_2)CO_2^-/H_2NC(H_2/D_2)CO_2^-$ on the rate of the HAT.

The large H/D KIE measured at pH 10.6, $k_H/k_D = 16.0 \pm 3.0$, clearly proves that the rate-determining step of reaction 7 is the HAT and not the electron-transfer reaction

$${}^\bullet CH_3 + :NH_2C(H_2/D_2)CO_2^-$$
$$\rightarrow CH_3^- + {}^{\bullet+}NH_2C(H_2/D_2)CO_2^- \quad (7')$$
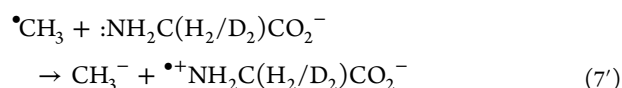
The large KIE is in accord with the large KIE reported for hydrogen atom abstraction from C−H groups.[13,28] However, the KIE for reaction 7 is considerably smaller than that reported for the reaction of H atoms with methanol and acetate.[13] This is somewhat surprising as k_7 is considerably smaller than the rate constants of the reactions of H$^\bullet$ atoms with methanol and acetate, and the KIE for the reactions of H$^\bullet$ atoms increases with the decrease in the rate constants of the reactions.[13] One would expect a similar behavior for the reactions of $^\bullet CH_3$ and H$^\bullet$ as the bond energies H−H and H−CH$_3$ are similar.[29,30] Alternatively, it might be suggested that some of the radicals react via reaction 7′, as happens for the $^\bullet$OH radicals,[12] and that the KIE for reaction 7 is considerably larger than 16. As $^\bullet CH_3$ radicals are weaker oxidizing agents, one expects that $k_7/k_{7'}$ for $^\bullet CH_3$ radicals will be larger than that for $^\bullet$OH radicals as the radical $H_2NC^\bullet(H/D)CO_2^-$ is considerably more stable than $^{\bullet+}NH_2C(H_2/D_2)CO_2^-$.[12]

Finally the large H/D KIE clearly points out that the HAT involves tunneling.[31] For the slow reaction, e.g., reaction 7, clearly tunneling accelerates the rate constants at least by an order of magnitude, assuming that tunneling does not contribute

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to deuterium abstraction reactions. This conclusion is surprising as the valence bond calculated activation energies, $\Delta E^{\#}_{VB}$, correlate linearly with the experimental activation energies, $\Delta G^{\#}_{exp}$ for HAT reactions.[32]

■ CONCLUSIONS

1. Methyl radicals react with glycine via a mechanism that involves, at least mainly, a H$^{\bullet}$ abstraction from the methylene group, as pointed out by the large KIE, and not, or only some, an e^{-} abstraction from the nitrogen amine.

2. The rate of this reaction is pH-dependent, increasing by several orders of magnitude above the last pK_a of glycine. Thus, probably even at physiological pH, that is, ca. 3 pH units below the pK_a of amino acids, alkyl radicals react with the alkaline form of amino acids present in the medium.

■ AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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