Biological Enolates: Generation and Stability of the Enolate of N-Protonated Glycine Methyl Ester in Water

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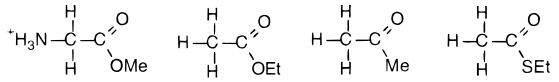
We report the formation of the amino acid ester enolate **2** by base-catalyzed deprotonation of N-protonated glycine methyl ester **1** that is *easily* followed at 25 °C and neutral pD and an estimated value of 21 for the pK_a for the α-protons of this simple amino acid derivative.



1 **3** **4** **5**

Amino acids are the building blocks for proteins, and their enolates are putative intermediates of nonenzymatic racemization of the α-carbon during chemical syntheses of peptides via activated amino acid esters[3] and of enzyme-catalyzed racemization of amino acids.[4] Despite the importance of these enolates in chemistry and biology, there have been few quantitative studies of their stability in water. Rate constants for their formation under extreme conditions have been determined in studies of α-hydrogen exchange or racemization reactions of amino acids and peptides at high temperatures[5−7,9] or in the presence of high concentrations of H_3O^+ [8,9] or HO^-.[10,11] *However, there are no reliable estimates of the pK_a values for the α-protons of amino acids or their derivatives.*[12]

The partial 500 MHz NMR spectra in Figures 1A−C show that deprotonation of **1** in D_2O resulting in the formation of **1-D** *can* (!!) be monitored at room temperature (25 °C) and neutral pD (7.4, 80 mM potassium phosphate, $I = 1.0$ KCl).[13a] Deuterium exchange results in a decrease in the area of the singlet at 3.921 ppm for the α-CH_2 group of **1** and the appearance of a triplet at 3.907 ppm ($J = 2.5$ Hz) for the α-CHD group.[14−16] The hydrolysis of **1** to form glycine is 10-times faster than exchange of the first α-hydrogen for deuterium, and the glycine obtained after completion of the hydrolysis reaction (>10 half-times) is enriched with 0.10 atom of deuterium (Figure 1D).

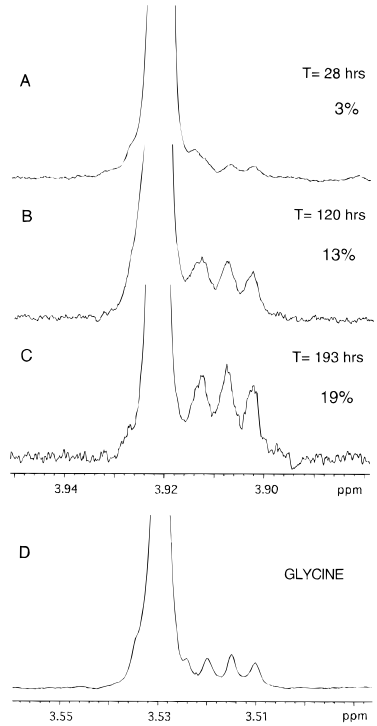
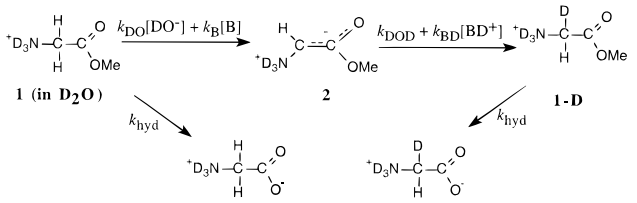


Figure 1. Representative partial ^{1}H NMR spectra at 500 MHz in water of **1** (Figures 1A−C) obtained during its reaction in D_2O at 25 °C and pD = 7.4 (80 mM potassium phosphate, $I = 1.0$ KCl), and a representative partial spectrum of glycine from the hydrolysis of **1** under the same conditions (Figure 1D). The fraction of monodeuteriated **1** or glycine is indicated at the top right of the appropriate spectrum.

Scheme 1



Two methods were used to determine values for the rate constant (k_{ex}, s^{-1}) for exchange of the first α-proton of **1** for deuterium. (1) Values of k_{ex} were determined as the slopes of linear semilogarithmic plots of reaction progress against time.[17a] (2) Values of k_{ex} were determined according to eq 1, where k_{hyd} (s^{-1}) is the rate constant for hydrolysis of **1** to form glycine (Scheme 1),[18] and f_D is the deuterium enrichment of glycine determined after ten halftimes for the hydrolysis reaction of **1**.[19]

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(12) Values of 15−17 for the pK_a of the α-proton of amino acids in water at pH 7.6 and 139 °C were calculated as p$K_a = -\log (k_{rac}/k_d)$, where k_{rac} is the observed first-order rate constant for racemization of the amino acid and $k_d = 6 \times 10^{10}$ M^{-1} s^{-1} is a rate constant for protonation of the amino acid enolate [ref 7a]. This calculation is incorrect, because it does not consider the basicity of the species which undergo the reversible proton transfer reaction with the amino acid.

(13) (a) The reactions of **1** (10−15 mM) in D_2O were quenched by adjusting the pD of the solution to 6 with concentrated DCl. ^{1}H NMR spectra at 500 MHz were determined as described in previous work.[14−16] (b) The pD of solutions which contained 3-chloroquinuclidine cation (80 mM potassium phosphate, pD 7.4) was adjusted to 13 with KOD, the amine was extracted into CCl_4, and the pD was readjusted to 7 with DCl.

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(18) The first-order rate constants for hydrolysis of **1** to form glycine and methanol were determined by ^{1}H NMR by following the disappearance of the signal for the methyl protons of **1**.

(19) The small estimated secondary deuterium isotope effect ($k_H/k_D = 0.96$) on the hydrolysis of monodeuteriated **1** will not have a significant effect on the final deuterium enrichment of glycine.[15]

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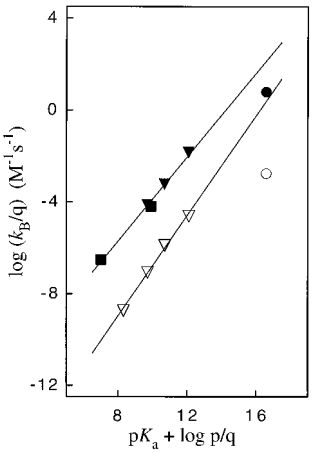

Figure 2. Brønsted correlations for deprotonation of **1** (filled symbols) and ethyl acetate (open symbols) in D_2O at 25 °C and $I = 1.0$ (KCl). pK_{BD} values are the apparent pK_a values of the catalysts in D_2O under the experimental conditions. Key: triangle, substituted quinuclidines; squares, phosphate dianion (pK_{BD} = 7.0) and hexafluoroisopropoxide anion (pK_{BD} = 9.9); circles, DO^-.

The values of k_{ex} determined by these methods agree to better

$$k_{ex} = k_{hyd}\left[\frac{f_D}{1 - f_D}\right] \qquad (1)$$

than 10%. Values of k_{ex} were generally determined by the first method. The second method was used for reactions in the presence of 3-chloroquinuclidinium ion, when it proved impossible to resolve the signals for the α-CH_2 protons of **1** and of this buffer acid.[13b]

A value of $k_{DO} = 6.0$ M^{-1} s^{-1} for DO^--catalyzed exchange of the first α-proton of **1** for deuterium was determined from the slope of a linear plot of k_o/f_{ND3+} against [DO^-] for reactions at pD 7.0, 7.4, and 8.0 (phosphate buffer), where k_o is obtained by extrapolation of the values of k_{ex} on a buffer catalysis plot to zero concentration of phosphate buffer and f_{ND3+} is the fraction of substrate present in the reactive N-protonated form. Values of k_B (M^{-1} s^{-1}) for general base catalysis[20] of deuterium exchange were determined as the slopes of plots of k_{ex}/f_{ND3+} against the concentration of the basic form of the buffer. Figure 2 shows Brønsted correlations of k_B (M^{-1} s^{-1}) for deprotonation of **1** and **3** (ethyl acetate).[15] These data allow a thorough analysis of the rate and equilibrium constants for formation of the enolate (**2**) of a simple amino acid derivative.

(1) The second-order rate constant $k_{DO} = 6.0$ M^{-1} s^{-1} for deprotonation of **1** is 20-fold larger than $k_{DO} = 0.3$ M^{-1} s^{-1} for deprotonation of acetone (**4**).[21] The relatively fast deprotonation of **1** reflects the large polar stabilization of **2** by interactions with the α-NH_3^+ group, and the large (*ca* 80%)[22] fractional expression of this polar effect at the transition state for formation of the enolate.[23]

(2) The 3500-fold difference in the values of k_{DO} for deprotonation of **1** (6.0 M^{-1} s^{-1}) and **3** (1.7×10^{-3} M^{-1} s^{-1})[15] requires that the pK_a for **1** be ≥3.5 (Δ log k_{DO}) units smaller than the pK_a of 25.6 for **3** (eq 2).[15] A pK_a of 25.6−4.4 ≈ 21

$$pK_a^{\,1} = pK_a^{\,3} - \Delta \log\left(\frac{k_{HO}}{k_{HOH}}\right) \qquad (2)$$

for the α-protons of **1** was calculated from eq 2. This calculation assumes similar solvent deuterium isotope effects on LO^--catalyzed deprotonation of **1** and **3** and that 80% of the effect of the α-NH_3^+ group on enolate stability is expressed in the value of k_{HO} for HO^--catalyzed deprotonation of **1** and 20% of the effect is expressed in the value of k_{HOH} for protonation of **2** by water.[22,24]

(3) The value of $\beta = 1.09$ for general base-catalyzed formation of the "liberated" enolate of ethyl acetate[15] suggests that proton transfer is complete prior to the rate-determining step for this reaction, and it is consistent with the conclusion that this rate-limiting step is diffusional separation of a complex between the enolate ion and the general acid.[15] By contrast, the value of $\beta = 0.91$ for the reaction of **1** shows that the rate of formation of **2** is limited by proton transfer from **1** to general base catalysts.

(4) The α-protons of **1** (pK_a ≈ 21) have roughly the same acidity as the corresponding protons for the thiol ester **5** (pK_a = 21)[14] and mandelic acid (pK_a = 22.0).[25] Therefore, the thermodynamic barrier to deprotonation of carbon will be similar for catalysis of deprotonation of enzyme-bound thiol esters, mandelic acid, and amino acids, provided the amino acid is bound in the N-protonated form and the negative charge at the carboxylate ion is neutralized by proton transfer or interaction with an enzyme-bound metal ion.[17,27]

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(20) For reactions at pD 7−8: The apparent second-order rate constants determined for phosphate-buffer-catalyzed deuterium exchange at several different ratios of the acidic and basic form of the buffer showed that catalysis is solely by the basic form of the buffer and that there is no detectable general acid catalysis. The rate constants k_{DO} and k_B correspond to the microscopic rate constants for deprotonation of **1** and **3** to form "liberated" enolate ions.[14,15]

(21) Calculated from $k_{HO} = 0.22$ M^{-1} s^{-1} for deprotonation of acetone in H_2O [Chiang, Y.; Kresge, A. J.; Morimoto, H.; Williams, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 3981−3982] and a solvent deuterium isotope effect of 1.46 on k_{HO} [Pocker, Y. *Chem. Ind.* **1959**, 1383−1384].

(22) The value of 80% was calculated as the ratio (Δ log k_{HO}/Δ log K_a) of the effects of an α-pyridinio substituent on the rate and equilibrium constants for deprotonation of acetophenone using values of $k_{HO} = 0.25$ and 1.8×10^5 M^{-1} s^{-1}, respectively, for acetophenone[26,28a] and α-pyridinioacetophenone[26] and pK_a values of 18.3[28a] and 10.9,[28b] respectively, for these two carbon acids. The fractional expression of the α-NH_3^+ substituent effect on k_{HO} for deprotonation of ethyl acetate is assumed to be the same as for this α-pyridinio substituent effect.

(23) By contrast, only 40% of enolate stabilization by "resonance" substituent effects is expressed at the transition state for transfer of α-carbonyl protons to hydroxide ion.[15] The explanation for this and related imbalances in the expression inductive and resonance substituent effects has been discussed in detail by Bernasconi [Bernasconi, C. F. *Adv. Phys. Org. Chem.* **1992**, *27*, 119−238].

(24) These proton transfer reactions are significantly uphill thermodynamically so that greater than 50% of the α-NH_3^+ substituent effect will be expressed at the transition state for proton transfer. Values of 60 and 100% as upper and lower limits for the uncertainty in this fraction set upper and lower limits of 19.7 and 22.1 for the pK_a for deprotonation of **1**.

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