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Formation and Stability of Peptide Enolates in Aqueous Solution

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Abstract: Second-order rate constants k_{DO} (M^{-1} s^{-1}) were determined in D_2O for deprotonation of the N-terminal α-amino carbon of glycylglycine and glycylglycylglycine zwitterions, the internal α-amino carbon of the glycylglycylglycine anion, and the acetyl methyl group and the α-amino carbon of the *N*-acetylglycine anion and *N*-acetylglycinamide by deuterioxide ion. The data were used to estimate values of k_{HO} (M^{-1} s^{-1}) for proton transfer from these carbon acids to hydroxide ion in H_2O. Values of the pK_a for these carbon acids ranging from 23.9 to 30.8 were obtained by interpolation or extrapolation of good linear correlations between log k_{HO} and carbon acid pK_a established in earlier work for deprotonation of related neutral and cationic α-carbonyl carbon acids. The α-amino carbon at a N-protonated N-terminus of a peptide or protein is estimated to undergo deprotonation about 130-fold faster than the α-amino carbon at the corresponding internal amino acid residue. The value of k_{HO} for deprotonation of the N-terminal α-amino carbon of the glycylglycylglycine zwitterion (pK_a = 25.1) is similar to that for deprotonation of the more acidic ketone acetone (pK_a = 19.3), as a result of a lower Marcus intrinsic barrier to deprotonation of cationic α-carbonyl carbon acids. The cationic NH_3^+ group is generally more strongly electron-withdrawing than the neutral NHAc group, but the α-NH_3^+ and the α-NHAc substituents result in very similar decreases in the pK_a of several α-carbonyl carbon acids.

Introduction

There have been many qualitative studies of the carbon deprotonation of L-amino acid residues in peptides and proteins. For example, racemization of amino acid residues and exchange of the α-amino proton for labeled hydrogen from solvent have both been observed during the hydrolysis of proteins in acidic[1] and basic[1,2] solution. Amino acid residues at proteins have been shown to racemize faster than amino acid monomers, and the relative rates of racemization of different amino acid residues at proteins have been reported.[2,3] Finally, the levels of accumulation of D-aspartic acid in the brain,[4] tooth enamel,[5,6] bones,[7] and lens protein[8] have been determined and shown to provide a rough measure of the age of these tissues.

By comparison, there have been no quantitative determinations of the carbon acid pK_a of the α-amino protons of amino acid residues at peptides or proteins. These carbon acidities are required for any full characterization of the chemical reactivity of these important compounds. They are relevant to the mechanism of racemization of amino acid residues in tissue,[4−8] fossils,[9] marine sediments,[10] and agricultural produce.[11] They are also of significant chemical interest, because of the insight that they would provide into the effect of the different functional groups present in peptides and proteins on the acidity of α-carbonyl protons.

We have developed convenient ^1H NMR methods to determine rate constants for deprotonation of simple carbon acids in D_2O.[12,13] We have established several simple relationships between rate constants for these proton-transfer reactions catalyzed by buffer bases and deuterioxide ion, which can be used to provide reliable estimates of carbon acid pK_a's in water.[12−19] We report here second-order rate constants k_{DO} (M^{-1} s^{-1}) for abstraction by deuterioxide ion of various α-carbonyl

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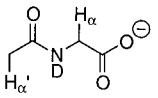
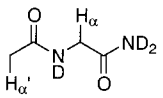
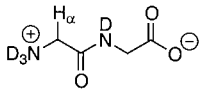
protons at the glycine derivatives **1** and **2** and at the glycine peptides **3** and **4**. The extensive relationships[12,16,17] between the kinetic (k_{DO}) and the thermodynamic (pK_a) acidity of a broad range of simple carbon acids have been used to obtain reliable pK_a's for various α-carbonyl protons at **1**−**4**. The data show that, neglecting the local effects of conformation and microenvironment, deprotonation of the α-amino carbon at a N-protonated N-terminus of a peptide or protein is about 130-fold faster than deprotonation of the α-amino carbon at the corresponding internal amino acid residue. They also allow an interesting comparison of the effect of α-NH_3^+ and α-NHAc substituents on the acidity of α-carbonyl protons, which may be relevant to the mechanism of enzymatic catalysis of formation of α-N(C═O) substituted carbanions.



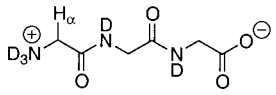
1-H_α
1-H_α'



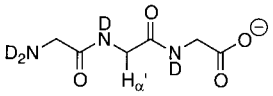
2-H_α
2-H_α'



3-H_α^+



4-H_α^+



4-H_α'

Experimental Section

Materials. *N*-Acetylglycine, *N*-acetylglycinamide, quinuclidine hydrochloride, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), 2,2,2-trifluoroethanol-d_3 (99.5% D), and KOD (40 wt %, 98+% D) were purchased from Aldrich. Glycylglycine (GlyGly) and glycylglycylglycine (GlyGlyGly) were purchased from Fluka. Deuterium oxide (99.9% D) and deuterium chloride (35% w/w, 99.5% D) were purchased from Cambridge Isotope Laboratories. Quinuclidine hydrochloride was recrystallized from ethanol. All other organic and inorganic chemicals were reagent grade and were used without further purification.

Preparation of Solutions. The acidic protons of substrates and buffer components were generally exchanged for deuterium before final preparation of solutions of these compounds in D_2O.[13] HFIP was dissolved directly in D_2O (99.9% D), which introduced <1 atom % of protium into this solvent.

Pyrophosphate buffers were prepared by dissolving the basic form and KCl in D_2O followed by the addition of DCl to give the desired acid/base ratio at $I = 1.0$ (KCl). Quinuclidine, HFIP, and trifluoroethoxide-d_2 buffers were prepared by dissolving the acidic form and KCl in D_2O followed by addition of KOD to give the desired acid/base ratio at $I = 1.0$ (KCl).

Solution pD was determined at 25 °C using an Orion model 720A pH meter equipped with a Radiometer GK2321C combination electrode. Values of pD were obtained by adding 0.40 to the observed reading of the pH meter.[20] The concentration of deuterioxide ion at any pD was calculated from eq 1, where $K_w = 10^{-14.87}$ is the ion product of D_2O at 25 °C[21], and $\gamma_{OL} = 0.79$ is the apparent activity coefficient of lyoxide ion determined for the particular electrode under our experimental conditions.[12]

$$[DO^-] = \frac{10^{pD-pK_w}}{\gamma_{OL}} \quad (1)$$

The apparent pK_a of pyrophosphate in D_2O at 25 °C and $I = 1.0$ (KCl), given by $pK_{BD} = pD - \log([B]/[BD^+])$, was determined as the observed pD of a gravimetrically prepared solution of 0.05 M buffer for which $[B] = [BD^+]$. Apparent pK_a's of $pK_{BD} = 8.81$ for the terminal amino group of GlyGly (**3**) and $pK_{BD} = 8.68$ for the terminal amino group of GlyGlyGly (**4**) in D_2O at 25 °C and $I = 1.0$ (KCl) were determined by potentiometric titration of a 10 mM solution of the substrate with KOD.[22]

^{1}H NMR Spectroscopy. ^{1}H NMR spectra at 500 MHz were recorded in D_2O at 25 °C on a Varian Unity Inova 500 spectrometer. Spectra were recorded with a sweep width of 2600 Hz, a 90° pulse angle, an acquisition time of 6 s, and zero-filling of the data to 64 K. Relaxation times T_1 were determined using 10 mM solutions of substrate at $I = 1.0$ (KCl). The relaxation delay between pulses was 10−20-fold greater than the longest T_1 for the protons of interest. Chemical shifts are reported relative to HOD at 4.67 ppm. Baselines were subjected to a first-order drift correction before determination of integrated peak areas.

Deuterium Exchange Reactions. All reactions were carried out in D_2O at 25 °C and a constant ionic strength of 1.0 maintained with potassium chloride. The deuterium exchange reactions of *N*-acetylglycinamide (**2**) were initiated by mixing solutions of the substrate and buffer at the same ionic strength (1.0, KCl) to give a final substrate concentration of 10 mM. The deuterium exchange reactions of GlyGly (**3**) and GlyGlyGly (**4**) were initiated by mixing solutions of the substrate and buffer at the same pD and ionic strength (1.0, KCl) to give a final substrate concentration of 10 mM. The deuterium exchange reactions of *N*-acetylglycine anion (**1**) in alkaline D_2O were initiated by mixing solutions of the anion of the substrate (prepared by neutralization of the substrate with 1 equiv of KOD) and KOD at the same ionic strength (1.0, KCl) to give a final substrate concentration of 10 mM.

A slow decrease in the solution pD was observed during the deuterium exchange reactions of **2** and **4** because of the formation of D_3O^+ from the competing hydrolysis reactions of these amides. The pD of the reaction mixture was monitored closely and was maintained within 0.05 units of the initial value by the periodic addition of small aliquots of 2.9 M KOD.

At timed intervals, a 0.6 mL aliquot was withdrawn from the reaction mixture, and the pD was adjusted to 7−8 by the addition of concentrated DCl and 50 μL of 0.4 M phosphate buffer (pD 7−8) in D_2O. These aliquots were either analyzed directly by ^{1}H NMR spectroscopy or frozen for analysis at a later time. The reported chemical shifts correspond to those for the quenched reaction mixtures at pD 7−8.

The exchange for deuterium of the first α-methyl protons **1-H_α′** and **2-H_α′** was followed by monitoring the disappearance of the singlets at 2.016 and 2.058 ppm, respectively, due to the α-CH_3 groups of the substrates and the appearance of well-resolved triplets due to the α-CH_2D groups of the products by ^{1}H NMR. The latter signals exhibit upfield shifts from the singlets of 0.014 ppm and coupling between the remaining α-protons and the α-deuterium ($J_{HD} = 2.5$ Hz). These deuterium isotope shifts and H−D couplings are similar to those observed in our earlier work.[12−14,16,17,19,23]

The exchange for deuterium of the first α-methylene protons **1-H_α**, **2-H_α**, **3-H_α^+**, **4-H_α^+**, and **4-H_α′** was followed by monitoring the disappearance of the singlets at 3.716, 3.885, 3.843, 3.874, and 4.020 ppm, respectively, due to the α-CH_2 groups of the substrates and the appearance of signals due to the α-CHD groups of the products by ^{1}H NMR. The latter signals exhibit upfield shifts from the singlets of 0.016 ppm for **1-H_α**, **2-H_α**, and **4-H_α′**, and 0.014 ppm for **3-H_α^+** and **4-H_α^+**. Well-resolved triplets were observed for α-CHD groups at **1** and **2** because there is a relatively large coupling between the remaining

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α-proton and the α-deuterium (J_{HD} = 2.5 Hz). However, the H−D coupling constants for α-CHD groups at **3** and **4** are smaller so that these signals appear as either a poorly resolved triplet or a broad singlet (see Results).

$$R = \frac{A_{CH_3}}{A_{CH_3} + \frac{A_{CH_2D}}{2}} \quad (2)$$

$$R = \frac{A_{CH_2}}{A_{CH_2} + A_{CHD}} \quad (3)$$

Values of *R*, which is a measure of the progress of the deuterium exchange reaction,[24a] were calculated according to eq 2 for the deuterium exchange reactions of α-methyl groups, or eq 3 for the deuterium exchange reactions of α-methylene groups. In these equations, A_{CH_3} and A_{CH_2} are the integrated areas of the singlets due to the α-CH_3 and α-CH_2 groups of the substrate, and A_{CH_2D} and A_{CHD} are the integrated areas of the triplets due to the α-CH_2D and α-CHD groups of the products.

$$\ln R = -k_{obsd}t \quad (4)$$

The exchange reactions of the α-methyl protons **1**-H_α' and **2**-H_α' were followed during exchange for deuterium of up to 14 and 7%, respectively, of the first α-proton of the methyl group. The exchange reactions of the α-methylene protons **1**-H_α, **2**-H_α, **3**-H_α^+, **4**-H_α^+, and **4**-H_α' were followed during exchange for deuterium of up to 40, 95, 30, 60, and 90%, respectively, of the first α-proton of the methylene group. Semilogarithmic plots of reaction progress (*R*, eqs 2 and 3) against time according to eq 4 were linear with negative slopes equal to k_{obsd} (s^{-1}), where k_{obsd} is the rate constant for the exchange of a *single* α-proton of the methyl or methylene group of the substrate. The values of k_{obsd} have been shown in our earlier work to be reproducible to ±10%.[17,19]

Results

Figure 1 shows representative ^{1}H NMR spectra at 500 MHz of GlyGly (**3**) during the exchange for deuterium of the first α-proton **3**-H_α^+ in the presence of 20 mM pyrophosphate buffer (pD = 9.2) in D_2O at 25 °C and *I* = 1.0 (KCl), obtained after quenching the reaction mixture to pD 7−8. Deuterium exchange leads to disappearance of the singlet at 3.843 ppm due to the N-terminal α-CH_2 group and the appearance of an upfield broad triplet at 3.829 ppm due to the α-CHD group of the product, in which the remaining α-proton is coupled to the α-deuterium. Figure 2 shows representative ^{1}H NMR spectra at 500 MHz of GlyGlyGly (**4**) during exchange for deuterium of the first α-protons **4**-H_α^+ and **4**-H_α' in the presence of 0.10 M quinuclidine buffer (pD = 11.8) in D_2O at 25 °C and *I* = 1.0 (KCl), obtained after quenching the reaction mixture to pD 7−8. Deuterium exchange leads to disappearance of the singlets at 3.874 and 4.020 ppm due to the N-terminal and internal α-CH_2 groups, respectively, and the appearance of broad signals at 3.860 and 4.004 ppm due to the corresponding α-CHD groups of the products.

The deuterium isotope effects on the ^{1}H chemical shifts for the α-methylene protons of **3** and **4** (0.014−0.016 ppm) are similar to those observed previously for other glycine derivatives.[17,19] However, the H−D coupling constants for α-CHD groups at **3** and **4** are smaller than J_{HD} = 2.5 Hz observed for other monodeuterated α-methyl or α-methylene groups,[12−17] so that these signals appear as either a poorly resolved triplet (Figure 1) or a broad singlet (Figure 2). The well-resolved triplets for α-CH_2D and α-CHD groups at **1** and **2** (not shown) that appear during the deuterium exchange reactions of these compounds resemble the triplets observed in studies of the deuterium exchange reactions of related α-carbonyl carbon acids.[12−19]

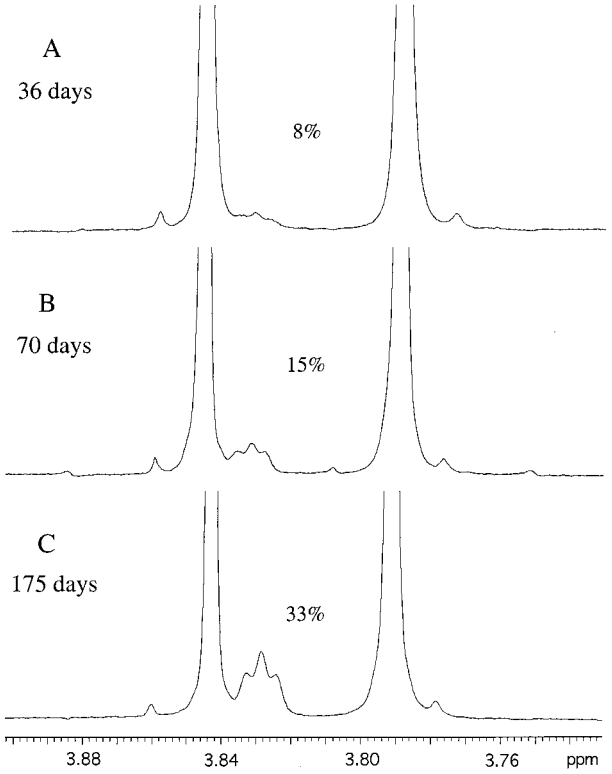

Figure 1. Representative ^{1}H NMR spectra at 500 MHz of GlyGly **3** during exchange for deuterium of 8−33% of the first α-proton **3**-H_α^+ in the presence of 20 mM pyrophosphate buffer (pD 9.2) in D_2O at 25 °C and *I* = 1.0 (KCl), obtained after quenching the reaction mixture to pD 7−8. Deuterium exchange results in loss of the singlet at 3.843 ppm due to the N-terminal α-CH_2 group and appearance of an upfield broad triplet at 3.829 ppm due to the α-CHD group of the product.

Exchange of α-Methyl Protons. The deuterium exchange reactions of the α-methyl protons **1**-H_α' and **2**-H_α' in D_2O at 25 °C and *I* = 1.0 (KCl) were followed by monitoring the appearance of the α-CH_2D group of the product by ^{1}H NMR. First-order rate constants k_{obsd} (s^{-1}) for the exchange for deuterium of a *single* proton of the α-methyl group were determined as the slopes of semilogarithmic plots of reaction progress *R* (eq 2) against time (not shown). The reaction of the α-CH_3 group of the substrate to give the product containing an α-CH_2D group occurs 3 times as fast as the exchange of a *single* proton of the α-CH_3 group, so that $k_{ex} = 3k_{obsd}$, where k_{ex} (s^{-1}) is the first-order rate constant for exchange for deuterium of the first proton of the α-CH_3 group of the substrate (Scheme 1A).[24a]

Table S1 of the Supporting Information gives values of $k_{ex} = (k_{ex})_o(s^{-1})$ for the solvent-catalyzed exchange for deuterium of the first α-methyl proton **1**-H_α' in the presence of various concentrations of deuterioxide ion. The plot (not shown) of $(k_{ex})_o$ against [DO^-] according to eq 5 is linear. The slope gives k_{DO}

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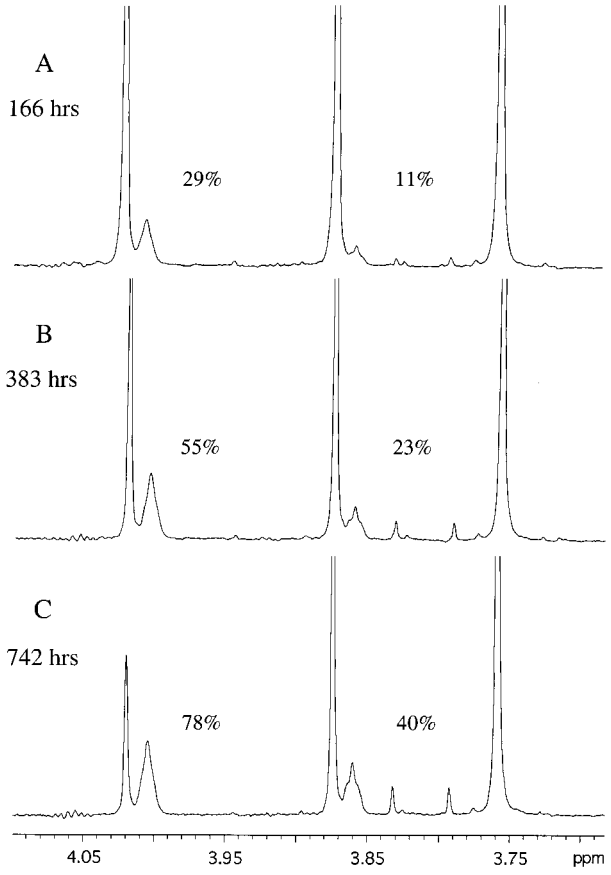
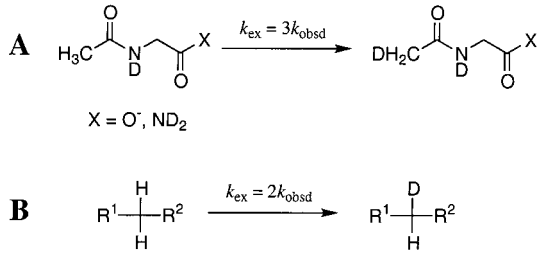


Figure 2. Representative ^{1}H NMR spectra at 500 MHz of GlyGlyGly **4** during exchange for deuterium of 11−40% of the first α-proton **4-H$_\alpha$$^+$** and 29−78% of the first α-proton **4-H$_\alpha$′** in the presence of 0.10 M quinuclidine buffer (pD 11.8) in D_2O at 25 °C and I = 1.0 (KCl), obtained after quenching the reaction mixture to pD 7−8. Deuterium exchange results in loss of the singlets at 3.874 and 4.020 ppm due to the N-terminal and internal α-CH_2 groups, respectively, and appearance of broad signals at 3.860 and 4.004 ppm due to the corresponding α-CHD groups of the products.

Scheme 1



= 4.5 × 10^{-6} M^{-1} s^{-1} (Table 1) as the second-order rate constant for exchange of the first α-methyl proton **1-H$_\alpha$′** catalyzed by DO^-. Figure 3 (▲) shows the pD-rate profile for the DO^--catalyzed exchange reaction of **1-H$_\alpha$′**. The solid line through the data was calculated from the value of k_{DO} using the logarithmic form of eq 5 in which [DO^-] is related to pD through eq 1.

Table S2 of the Supporting Information gives values of k_{ex} (s^{-1}) for the exchange for deuterium of the first α-methyl proton **2-H$_\alpha$′** in the presence of 20 and 50 mM trifluoroethoxide buffer at pD 12.6. The observation of essentially the same value of k_{ex} = 1.35 ± 0.03 × 10^{-7} s^{-1} for deuterium exchange at these two buffer concentrations shows that there is no significant buffer catalysis of exchange under these conditions so that k_{ex}

Table 1. Rate and Equilibrium Constants for Carbon Deprotonation of Amino Acid Derivatives and Peptides in Water at 25 °C (I = 1.0, KCl)

carbon acid	proton	k_{DO} (M^{-1} s^{-1})[a]	k_{HO} (M^{-1} s^{-1})[b]	pK_a[c]
1	H_α	1.9 × 10^{-6}	9.5 × 10^{-7}	30.8
	H_α′	4.5 × 10^{-6}	2.3 × 10^{-6}	30.3
2	H_α	2.6 × 10^{-3}	1.3 × 10^{-3}	23.9
	H_α′	2.1 × 10^{-5}	1.1 × 10^{-5}	29.1
3	H_α^+	3.5 × 10^{-2}	1.8 × 10^{-2}	26.7[d]
4	H_α^+	0.23	0.12	25.1
	H_α′	3.9 × 10^{-4}	2.0 × 10^{-4}	25.9

[a] Second-order rate constant for deprotonation of the carbon acid by deuterioxide ion in D_2O, determined by monitoring deuterium incorporation into the carbon acid by ^{1}H NMR. [b] Second-order rate constant for deprotonation of the carbon acid by hydroxide ion in H_2O, calculated from the value of k_{DO} using an estimated secondary solvent deuterium isotope effect of k_{DO}/k_{HO} = 2.0 [ref 34]. [c] pK_a for ionization of the carbon acid in water obtained as described in the text. [d] Calculated from the value of k_{HO} and the limiting value of $k_{HOH} = (k_{HOH})_{lim} = k_{reorg} \approx 10^{11}$ s^{-1} for protonation of the enolate by solvent water using eq 8 (see text).

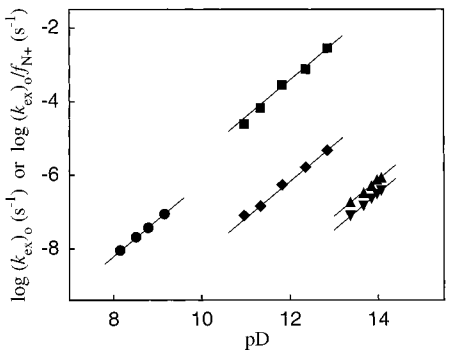


Figure 3. pD-Rate profiles of $(k_{ex})_o$ (s^{-1}) or $(k_{ex})_o/f_{N+}$ (s^{-1}) for the buffer-independent exchange for deuterium of the first proton of the α-methyl and α-methylene groups of glycine derivatives in D_2O at 25 °C and I = 1.0 (KCl). The solid lines through the data were calculated from the values of k_{DO} (M^{-1} s^{-1}, Table 1) using the logarithmic form of eq 5 for **1-H$_\alpha$**, **1-H$_\alpha$′**, and **4-H$_\alpha$′** or eq 6 for **3-H$_\alpha$$^+$** and **4-H$_\alpha$$^+$** (see text). (●) Data for **3-H$_\alpha$$^+$**. Values of f_{N+} were calculated from the solution pD and pK_{BD} = 8.81 for the terminal amino group of GlyGly in D_2O (25 °C, I = 1.0, KCl). (■) Data for **4-H$_\alpha$$^+$**. Values of f_{N+} were calculated from the solution pD and pK_{BD} = 8.68 for the terminal amino group of GlyGlyGly in D_2O (25 °C, I = 1.0, KCl). (◆) Data for **4-H$_\alpha$′**. (▲) Data for **1-H$_\alpha$′**. (▼) Data for **1-H$_\alpha$**.

= $(k_{ex})_o$. The value of k_{DO} = 2.1 × 10^{-5} M^{-1} s^{-1} (Table 1) for exchange of the first α-methyl proton **2-H$_\alpha$′** catalyzed by DO^- was calculated from this buffer-independent value of $(k_{ex})_o$ (s^{-1}) using eq 5.

$$(k_{ex})_o = k_{DO}[DO^-] \quad (5)$$

Exchange of α-Methylene Protons. The deuterium exchange reactions of the α-methylene protons **1-H$_\alpha$**, **2-H$_\alpha$**, **3-H$_\alpha$$^+$**, **4-H$_\alpha$$^+$**, and **4-H$_\alpha$′** in D_2O at 25 °C and I = 1.0 (KCl) were followed by monitoring the appearance of the α-CHD group of the product by ^{1}H NMR. First-order rate constants k_{obsd} (s^{-1}) for the exchange for deuterium of a *single* proton of the α-methylene group were determined as the slopes of semilogarithmic plots of reaction progress R (eq 3) against time (not shown). The reaction of the α-CH_2 group of the substrate to give the product containing an α-CHD group occurs 2 times as fast as the exchange of a *single* proton of the α-CH_2 group, so that k_{ex} = $2k_{obsd}$, where k_{ex} (s^{-1}) is the first-order rate constant for the exchange for deuterium of the first proton of the α-CH_2 group of the substrate (Scheme 1B).[17,24a]

Table S1 of the Suppporting Infomation gives values of $k_{ex} = (k_{ex})_o$ (s^{-1}) for the solvent-catalyzed exchange for deuterium of the first α-methylene proton **1-H$_\alpha$** in the presence of various concentrations of deuterioxide ion. The plot (not shown) of $(k_{ex})_o$ against [DO^-] according to eq 5 is linear. The slope gives $k_{DO} = 1.9 \times 10^{-6}$ M^{-1} s^{-1} (Table 1) as the second-order rate constant for exchange of the first α-methylene proton **1-H$_\alpha$** catalyzed by DO^-. Figure 3 (▼) shows the pD-rate profile for the DO^--catalyzed exchange reaction of **1-H$_\alpha$**. The solid line through the data was calculated from the value of k_{DO} using the logarithmic form of eq 5 in which [DO^-] is related to pD through eq 1.

Table S2 of the Supporting Information gives values of k_{ex} (s^{-1}) for the exchange for deuterium of the first α-methylene proton **2-H$_\alpha$** in the presence of 20 and 50 mM trifluoroethoxide buffer at pD 12.6. The observation of essentially the same value of $k_{ex} = 1.66 \pm 0.04 \times 10^{-5}$ s^{-1} for deuterium exchange at these two buffer concentrations shows that there is no significant buffer catalysis of exchange under these conditions so that $k_{ex} = (k_{ex})_o$. The value of $k_{DO} = 2.6 \times 10^{-3}$ M^{-1} s^{-1} (Table 1) for the exchange of the first α-methylene proton **2-H$_\alpha$** catalyzed by DO^- was calculated from this buffer-independent value of $(k_{ex})_o$ (s^{-1}) using eq 5.

Table S3 of the Supporting Information gives values of k_{ex} (s^{-1}) for the exchange for deuterium of the first α-methylene proton **3-H$_\alpha$**$^+$ in the presence of 20 mM pyrophosphate buffer in D_2O at pD 8.2−9.2. There is no significant catalysis of deprotonation of the α-amino carbon of N-protonated glycine methyl ester or betaine methyl ester, whose structures are similar to **3-H$_\alpha$**$^+$, by pyrophosphate buffer at this concentration.[17] Therefore, we expect that the values of k_{ex} reported in Table S3 for the deuterium exchange reaction of **3-H$_\alpha$**$^+$ are essentially equal to $(k_{ex})_o$ (s^{-1}) for solvent-catalyzed exchange. The plot (not shown) of $(k_{ex})_o/f_{N+}$ against [DO^-] according to eq 6 is linear, where f_{N+} is the fraction of the substrate present in the reactive N-protonated form, calculated from the pD and the apparent pK_a in D_2O of 8.81 for the terminal amino group of GlyGly (see Experimental Section). The slope gives $k_{DO} = 3.5 \times 10^{-2}$ M^{-1} s^{-1} (Table 1) as the second-order rate constant for exchange of the first α-methylene proton **3-H$_\alpha$**$^+$ catalyzed by DO^-. Figure 3 (●) shows the pD-rate profile for the DO^--catalyzed exchange reaction of **3-H$_\alpha$**$^+$. The solid line through the data was calculated from the value of k_{DO} using the logarithmic form of eq 6 in which [DO^-] is related to pD through eq 1.

$$\frac{(k_{ex})_o}{f_{N+}} = k_{DO}[DO^-] \qquad (6)$$

Table S4 of the Supporting Information gives values of k_{ex} (s^{-1}) for exchange for deuterium of the first α-methylene protons **4-H$_\alpha$**$^+$ and **4-H$_\alpha$**′ in the presence of 0.05−0.20 M HFIP buffer at pD 11.0 and in the presence of 0.05−0.30 M quinuclidine buffer at pD 11.3−12.9. There are only very small increases of ≤25% in k_{ex} (s^{-1}) for these reactions in the presence of increasing concentrations of these buffers. Table S4 gives the values of $(k_{ex})_o$ (s^{-1}) for the buffer-independent solvent-catalyzed deuterium exchange reactions of **4-H$_\alpha$**$^+$ and **4-H$_\alpha$**′ that were determined from short extrapolations of the values of k_{ex} to zero buffer concentration.

The values of $(k_{ex})_o$ for the deuterium exchange reaction of the α-methylene group at the N-terminal amino acid residue of **4** at pD ≫ pK_a for the terminal amino group remain nearly constant as the pD is increased from 11.0 to 12.9 (Table S4). This shows that **4-H$_\alpha$**$^+$ is the reactive form of the substrate for the DO^--catalyzed deuterium exchange reaction. The plot (not shown) of $(k_{ex})_o/f_{N+}$ against [DO^-] according to eq 6 is linear, where f_{N+} is the fraction of the substrate present in the reactive N-protonated form, calculated from the pD and the apparent pK_a in D_2O of 8.68 for the terminal amino group of GlyGlyGly (see Experimental Section). The slope gives $k_{DO} = 0.23$ M^{-1} s^{-1} (Table 1) as the second-order rate constant for exchange of the first α-methlyene proton **4-H$_\alpha$**$^+$ catalyzed by DO^-. Figure 3 (■) shows the pD-rate profile for the DO^--catalyzed exchange reaction of **4-H$_\alpha$**$^+$. The solid line through the data was calculated from the value of k_{DO} using the logarithmic form of eq 6 in which [DO^-] is related to pD through eq 1.

By contrast, the values of $(k_{ex})_o$ for the deuterium exchange reaction of the α-methylene group at the internal amino acid residue of **4** are directly proportional to [DO^-], which shows that **4-H$_\alpha$**′ is the reactive form of the substrate for this deuterium exchange reaction. The plot (not shown) of $(k_{ex})_o$ against [DO^-] according to eq 5 is linear. The slope gives $k_{DO} = 3.9 \times 10^{-4}$ M^{-1} s^{-1} (Table 1) as the second-order rate constant for the exchange of the first α-methylene proton **4-H$_\alpha$**′ catalyzed by DO^-. Figure 3 (◆) shows the pD-rate profile for the DO^--catalyzed exchange reaction of **4-H$_\alpha$**′. The solid line through the data was calculated from the value of k_{DO} using the logarithmic form of eq 5 in which [DO^-] is related to pD through eq 1.

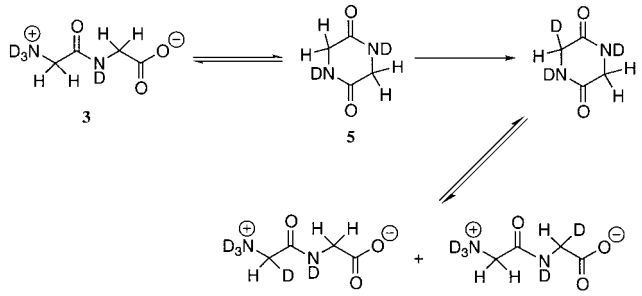
Discussion

In an earlier study of the deuterium exchange reactions of the α-methylene protons of GlyGlyGly, GlyGly, and glycine in D_2O at pD 13.3, monitored by ^1H NMR at 100 MHz, no deuterium exchange into glycine or GlyGly was detected after 21 h, but there was significant deuterium exchange into the internal amino acid residue of the tripeptide GlyGlyGly after 5 h.[25] We observe similar relative rates for these deuterium exchange reactions in this work. A second-order rate constant $k_{HO} = 7.7 \times 10^{-4}$ M^{-1} s^{-1} for the hydroxide-catalyzed racemization of *N*-acetyl-L-serine amide in water at 24 °C has been reported.[26] This is close to $k_{HO} = 1.3 \times 10^{-3}$ M^{-1} s^{-1} for deprotonation of *N*-acetylglycinamide **2-H$_\alpha$** by hydroxide ion at 25 °C that can be calculated from k_{DO} for the deuterium exchange reaction (Table 1). This suggests that the addition of an α-hydroxymethyl group has only a small effect on the rate constant for deprotonation of the α-amino carbon of this glycine derivative.

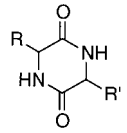
Mechanism of Deuterium Exchange. It has been suggested that the racemization and deuterium exchange reactions at the α-amino carbons of dipeptides may proceed by the reversible formation of diketopiperazine **5** (Scheme 2), because **5** would be more reactive toward carbon deprotonation than would the parent dipeptide.[27−30] However, there was no detectable (<5%) formation of 2,5-piperazinedione (**5**, R = R′ = H) during the deuterium exchange reactions of GlyGly (**3**) in D_2O at pD 8.2−

(25) Fridkin, M.; Wilchek, M.; Sheinblatt, M. *Biochem. Biophys. Res. Commun.* **1970**, *38*, 458−464.
(26) Bohak, Z.; Katchalski, E. *Biochemistry* **1963**, *2*, 228−237.
(27) Steinberg, S.; Bada, J. L. *Science* **1981**, *213*, 544−545.
(28) Mitterer, R. M.; Kriausakul, N. *Org. Geochem.* **1984**, *7*, 91−98.
(29) Smith, G. G.; Evans, R. C.; Baum, R. *J. Am. Chem. Soc.* **1986**, *108*, 7327−7332.
(30) Smith, G. G.; Baum, R. *J. Org. Chem.* **1987**, *52*, 2248−2255.

Scheme 2



9.2 and 25 °C, monitored by ^{1}H NMR (Scheme 2). In these reactions, there was deuterium incorporation only at the N-terminal α-amino carbon of GlyGly. This shows that deuterium exchange into GlyGly does *not* occur through a low concentration of **5** that is in rapid equilibrium with **3**, because the reversible formation of **5** would result in scrambling of the deuterium that is initially incorporated at the N-terminal α-amino carbon of GlyGly between the N-terminal and C-terminal positions (Scheme 2).



5

There is good evidence that the base-catalyzed exchange for deuterium of the α-carbonyl protons of simple aldehydes,[31] ketones,[31] thiolesters,[13] oxygen esters,[12] and amides[16] proceeds by a stepwise mechanism through an enolate intermediate when the enolate is sufficiently stable to exist for the time of a bond vibration (10^{-13} s).[32,33] We assume here the same stepwise mechanism for the DO^--catalyzed deuterium exchange reactions of **1**−**4**, because the rate constants k_{DO} (M^{-1} s^{-1}, Table 1) for these reactions are no more than 10-fold smaller than that for the stepwise deuterium exchange reaction of acetamide,[16] so that the lifetimes of the putative enolate intermediates should not be dramatically shorter than $1/k_{HOH} = 4 \times 10^{-10}$ s that was obtained for the enolate of acetamide.[16]

Carbon Acid p*K*$_a$'s. (1) "Neutral" α-Carbonyl Carbon Acids. Second-order rate constants k_{HO} (M^{-1} s^{-1}, Table 1) for the deprotonation of **1-H**$_\alpha$, **1-H**$_\alpha$′, **2-H**$_\alpha$, **2-H**$_\alpha$′, and **4-H**$_\alpha$′ by hydroxide ion in H_2O to form the corresponding enolates were calculated from the experimental values of k_{DO} (M^{-1} s^{-1}, Table 1) for deprotonation by deuterioxide ion and an estimated secondary solvent deuterium isotope effect of $k_{DO}/k_{HO} = 2.0$.[34] These rate constants define the relative kinetic acidity of these neutral carbon acids. The pK_a's for these α-carbonyl protons were estimated from the kinetic acidity given by k_{HO} (M^{-1} s^{-1}),

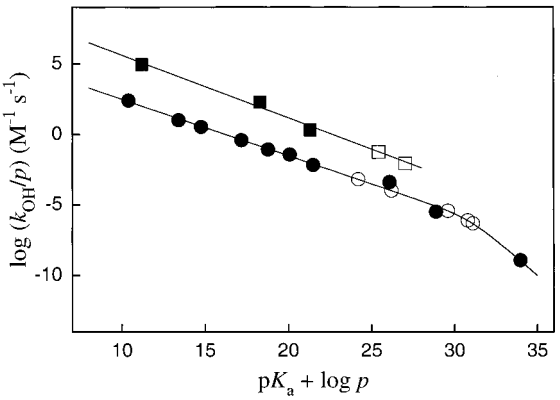
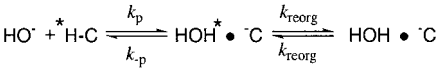
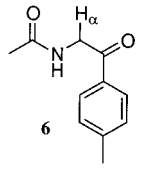


Figure 4. Rate-equilibrium correlations of rate constants, k_{HO} (M^{-1} s^{-1}), for deprotonation of monocarbonyl carbon acids by hydroxide ion with the pK_a of the carbon acid. The values of k_{HO} and pK_a were statistically corrected for the number of acidic protons p at the carbon acid. (●) Correlation of log(k_{HO}/p) for deprotonation of neutral aldehydes, ketones, esters, and acetamide by hydroxide ion, constructed using data from earlier work.[12,13,16,31,35a] Excluding the point for $CH_3CO_2^-$ (pK_a = 33.5), the data are correlated by log(k_{HO}/p) = 6.496 − 0.401(pK_a + log p). (■) Correlation of log(k_{HO}/p) for deprotonation of cationic ketones and esters.[17] The data are correlated by log(k_{HO}/p) = 10.044 − 0.444(pK_a + log p). (○) Data for deprotonation of **1-H**$_\alpha$, **1-H**$_\alpha$′, **2-H**$_\alpha$, **2-H**$_\alpha$′, and **4-H**$_\alpha$′. The pK_a's for these carbon acids (Table 1) were calculated with the assumption that the values of log k_{HO} for their deprotonation lie on the correlation line for deprotonation of neutral α-carbonyl carbon acids. (□) Data for deprotonation of **3-H**$_\alpha^+$ and **4-H**$_\alpha^+$ (Table 1).

Scheme 3



by interpolation (Figure 4, ○) of the excellent "biphasic" correlation between log k_{HO} and carbon acid pK_a for the deprotonation of a wide range of neutral monocarbonyl compounds by hydroxide ion that we have reported in earlier work (Figure 4, ●).[12,16] This correlation includes data for the *N*-acetyl substituted ketone **6-H**$_\alpha$ (pK_a = 14.45),[35a] whose structure is similar to the *N*-acetyl substituted α-carbonyl carbon acids **1-H**$_\alpha$ and **2-H**$_\alpha$.



6

The lower correlation shown in Figure 4 (●) shows an extended linear region with a slope of −0.40 up to p$K_a \approx 30$, with a downward break to a slope of −1.0 at the point where the rate-determining step for formation of the "free" enolate changes from k_p for deprotonation of the carbon acid ($k_{-p} < k_{reorg} \approx 10^{11}$ s^{-1}, Scheme 3) to k_{reorg} for reorganization of the surrounding solvent water ($k_{-p} > k_{reorg}$).[16] The change in the

$$(k_{HO}/p) = \frac{k_p k_{reorg}}{k_{-p} + k_{reorg}} \qquad (7)$$

rate-limiting step when $k_{-p} = k_{reorg}$ occurs at a carbon acid pK_a

(31) Keeffe, J. R.; Kresge, A. J. In *The Chemistry of Enols*; Rappoport, Z., Ed.; John Wiley and Sons: Chichester, 1990; pp 399−480.
(32) Jencks, W. P. *Acc. Chem. Res.* **1980**, *13*, 161−169.
(33) Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345−375.
(34) The secondary solvent deuterium isotope effect for deprotonation of these carbon acids by lyoxide ion is expected to be larger than $k_{DO}/k_{HO} = 1.46$ for deprotonation of acetone [Pocker, Y. *Chem. Ind.* **1959**, 1383−1384]. However, we have no evidence that it is close to the maximum value of $k_{DO}/k_{HO} = 2.4$ for deprotonation to form very unstable carbanions, for which isotope exchange is limited by solvent reorganization that exchanges the hydron of substrate with a labeled hydron from solvent.[43] Therefore, we use an intermediate value as the estimated secondary solvent deuterium isotope effect for deprotonation of the carbon acids studied in this work.

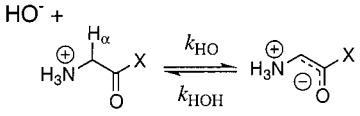
(35) (a) Chiang, Y.; Griesbeck, A. G.; Heckroth, H.; Hellrung, B.; Kresge, A. J.; Meng, Q.; O'Donoghue, A. C.; Richard, J. P.; Wirz, J. *J. Am. Chem. Soc.* **2001**, *123*, 8979−8984. (b) Chiang, Y.; Kresge, A. J.; Tang, Y. S.; Wirz, J. *J. Am. Chem. Soc.* **1984**, *106*, 460−462.

of 31. The solid line through the data in the lower correlation in Figure 4 (●) shows the fit of the data to eq 7, derived for Scheme 3, where $k_p = 10^{\{-0.401(pK_a + \log p) + 6.496\}}$ and $k_{-p} = 10^{\{0.599(pK_a + \log p) - 7.506\}}$ define the rate constants for the linear portion of the correlation up to pK_a = 28.4 for acetamide, and $k_{reorg} = 10^{11}$ s^{-1}.[16] The pK_a's for the neutral carbon acids **1-H$_\alpha$**, **1-H$_\alpha$′**, **2-H$_\alpha$**, **2-H$_\alpha$′**, and **4-H$_\alpha$′** reported in Table 1 were obtained by interpolation of this correlation (○). The estimated limits of uncertainty in the pK_a's for these carbon acids are ±0.5 units, which is the magnitude of the average deviation of the experimental pK_a's from the correlation line.

(2) "Cationic" Carbon Acids. Second-order rate constants k_{HO} (M^{-1} s^{-1}, Table 1) for the deprotonation of **3-H$_\alpha$**$^+$ and **4-H$_\alpha$**$^+$ by hydroxide ion in H_2O to form the corresponding enolates were calculated from the experimental values of k_{DO} (M^{-1} s^{-1}, Table 1) for the deprotonation by deuterioxide ion and an estimated secondary solvent deuterium isotope effect of $k_{DO}/k_{HO} = 2.0$.[34] These rate constants define the relative kinetic acidity of these cationic carbon acids. It has been shown that α-NH_3^+ and α-NMe_3^+ substituted α-carbonyl carbon acids undergo deprotonation by hydroxide ion 10^2-10^3-fold faster than a neutral α-carbonyl carbon acid of the same pK_a.[17] A similar large *kinetic* acidity is also observed here for **3-H$_\alpha$**$^+$ and **4-H$_\alpha$**$^+$. For example, the value of $k_{HO} = 0.12$ M^{-1} s^{-1} for deprotonation of **4-H$_\alpha$**$^+$ (Table 1) is similar to the statistically corrected value of $k_{HO} = 0.11$ M^{-1} s^{-1} for deprotonation of a single methyl group of acetone,[31,35b] but the pK_a of the amide **4-H$_\alpha$**$^+$ is expected to be substantially higher than the statistically corrected pK_a of 19.6 for a single methyl group of acetone.[31,35b] The much larger rate constants for deprotonation of cationic ketones,[24] esters,[17] and amides (this work) than for deprotonation of a neutral α-carbonyl carbon acid of the same pK_a require that there be a smaller Marcus intrinsic barrier for deprotonation of these cationic carbon acid substrates.[36] The explanation for the difference in the intrinsic barrier for these related reactions has been discussed in earlier work.[17,24]

Figure 4 (■) shows the correlation of log k_{HO} with carbon acid pK_a for deprotonation of a series of cationic ketones and esters. This correlation lies 2−3 log units above the correlation line for deprotonation of neutral carbon acids (Figure 4, ●). The data for these cationic carbon acids are correlated by log-$(k_{HO}/p) = 10.044 - 0.444(pK_a + \log p)$ (Figure 4, upper correlation line). Carbon acid pK_a's of 26.9 for **3-H$_\alpha$**$^+$ and 25.1 for **4-H$_\alpha$**$^+$ (Table 1) can be calculated by assuming that the values of k_{HO} for their deprotonation lie on the upper correlation line in Figure 4 (□).

Scheme 4

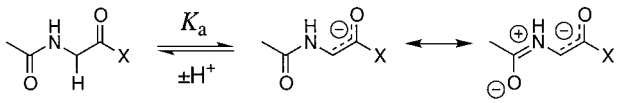


$$pK_a = pK_w + \log\left(\frac{k_{HOH}}{k_{HO}}\right) \qquad (8)$$

These pK_a's lie very close to the upper limits of p$K_a \le 26.7$ for **3-H$_\alpha$**$^+$ and p$K_a \le 25.9$ for **4-H$_\alpha$**$^+$ that can be calculated using eq 8, derived for Scheme 4, with the limiting value of $(k_{HOH})_{lim} = k_{reorg} \approx 10^{11}$ s^{-1} for protonation of the enolate by solvent water and pK_w = 14.[16] By comparison, lower limits of p$K_a \ge 23.4$ for **3-H$_\alpha$**$^+$ and p$K_a \ge 22.5$ for **4-H$_\alpha$**$^+$ can be estimated from k_{HO} (M^{-1} s^{-1}) for their deprotonation by hydroxide ion (Table 1) and $k_{HO} = 4.1$ M^{-1} s^{-1} for deprotonation of N-protonated glycine methyl ester (Scheme 4, X = OMe, pK_a = 21.0),[17] with the unlikely assumption that the greater carbon acidity of this cationic ester as compared to those of **3-H$_\alpha$**$^+$ (Scheme 4, X = N(D)$CH_2CO_2^-$) and **4-H$_\alpha$**$^+$ (Scheme 4, X = N(D)$CH_2CON(D)CH_2CO_2^-$) is expressed entirely in the rate constant k_{HO} for the deprotonation of the carbon acid. The uncertainty in the estimated pK_a's of 26.7 for **3-H$_\alpha$**$^+$ and 25.1 for **4-H$_\alpha$**$^+$ (Table 1) is larger than that (±0.5 units) estimated above for neutral carbon acids, because they were obtained by making relatively long extrapolations of a linear correlation of limited experimental data.

Substituent Effects on Carbon Acidity. Table 2 compares the effects of α-NHAc and α-NH_3^+ substituents on the carbon acidity of simple carbonyl compounds in aqueous solution. The addition of an α-NH_3^+ group results in a 4.8 unit decrease in the carbon acid pK_a of the α-methyl group of acetate anion and ethyl acetate, and a smaller 3.8 unit decrease in the pK_a of the α-methyl group of *N*-acetylglycine anion. By comparison, the addition of an α-NHAc group results in 4.7 and 4.4 unit decreases in the carbon acid pK_a of the α-methyl group of acetamide and 4-methylacetophenone, respectively, and a smaller 2.9 unit decrease in the pK_a of the α-methyl group of acetate anion. These data are consistent with the conclusion that α-NHAc and α-NH_3^+ groups result in similar changes in the carbon acidity of α-carbonyl protons. The 2 unit variation in the effects of α-NHAc and α-NH_3^+ groups on the carbon acidity of various α-carbonyl protons (Table 2) is consistent with the uncertainty of ±0.5 units for the individual carbon acid pK_a's.

Scheme 5

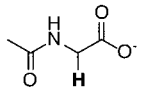
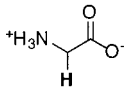
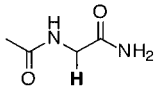
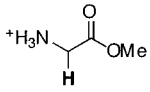
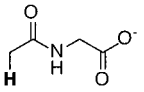
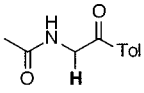
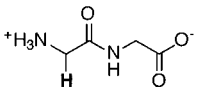


The larger inductive substituent constant $\sigma_I = 0.60$ for the NH_3^+ group as compared with $\sigma_I = 0.26$ for the NHAc group shows that polar interactions with NH_3^+ are usually more stabilizing than the corresponding interaction with an NHAc group.[37] By contrast, the observation here that α-NH_3^+ and α-NHAc groups result in *similar* decreases in the pK_a's for ionization of α-carbonyl carbon acids shows that these substituents provide *similar* stabilization of negative charge at a neighboring enolate carbon. In other words, the polar effect of the NHAc substituent is enhanced relative to that of the NH_3^+ substituent when these groups are *directly* attached to a charged atom. We suggest that placing an α-NHAc substituent at an enolate carbon results in an "adjustment" of the structure and charge distribution of the α-amide group toward a greater contribution of the zwitterionic resonance form (Scheme 5), because this maximizes the stabilization from interaction of neighboring charges of opposite sign. Such a localization of positive charge at the α-amide nitrogen should be at a maximum when the neighboring atom carries a full negative charge,

(36) Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 891−899. Kresge, A. J. *Chem. Soc. Rev.* **1974**, *2*, 475−503.

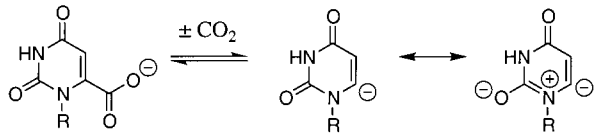
(37) Hine, J. *Structural Effects on Equilibria in Organic Chemistry*; Wiley: New York, 1975; p 98.

Table 2. Effects of α-NHAc and α-NH_3^+ Substituents on the Carbon Acidity of Simple Carbonyl Compounds in Water[a]

	Carbon Acid Substituent			
	α-NHAc		α-NH_3^+	
Carbon Acid	$pK_a + \log p$ [b]	$\Delta(pK_a + \log p)$[c]	Carbon Acid	$pK_a + \log p$ [b]	$\Delta(pK_a + \log p)$[c]
	34.0 [d]			34.0 [d]	
		2.9			4.8
	31.1			29.2 [e]	
	28.9 [d]			26.1 [f]	
		4.7			4.8
	24.2			21.3 [e]	
	19.2 [g]			30.8	
		4.4			3.8
	14.8 [g]			27.0	

[a] Data from Table 1, unless noted otherwise. [b] pK_a for ionization of the carbon acid in water at 25 °C with a statistical correction for the number of acidic protons *p*. [c] Effect of the α-substituent on carbon acidity. [d] Data from ref 16. [e] Data from ref 17. [f] Data from ref 12. [g] Data from ref 35a.

Scheme 6



OMP

because this results in the largest electrostatic stabilization for a given redistribution of charge at the α-amide nitrogen.

Other Relevance. The α-NHAc group, which is normally substantially less polar and less electron-withdrawing than the α-NH_3^+ group, has roughly the same effect as an α-NH_3^+ group on enolate stability (Table 2), and might provide an unexpectedly large stabilization of other carbanions. The decarboxylation of orotidine 5′-monophosphate (OMP) is catalyzed by OMP decarboxylase (Scheme 6, R = ribose 5′-phosphate). The major obstacle to enzymatic catalysis of the direct decarboxylation of OMP to form a vinylic carbanion[38] is the very high basicity of simple vinyl carbanions, whose conjugate acids are of $pK_a \approx 44$.[39] However, this basicity should be reduced substantially by a stabilizing interaction of the negative charge with the α-NRC(O)R′ group (Scheme 6), and this will favor the stepwise reaction mechanism. This carbanion stabilization because of the α-NRC(O)R′ group may be further enhanced by catalysis at an active site of low dielectric constant, because this will favor the development of intramolecular electrostatic interactions at the zwitterionic valence bond resonance form of this carbanion (Scheme 6).[17,40] The failure of X-ray crystallographic analysis to identify any acidic active site residues that are situated to protonate either the O-2 or the O-4 carbonyl oxygen of OMP is consistent with the formation of the carbanion intermediate shown in Scheme 6.[41]

The second-order rate constant k_{DO} for the DO^--catalyzed exchange for deuterium of the N-terminal α-amino proton **4-H_α^+** ($k_{DO} = 0.23\ M^{-1}\ s^{-1}$, Table 1) is similar to the rate constant for the corresponding exchange reaction of acetone.[35b] The large effect of the α-NH_3^+ group on kinetic and thermodynamic carbon acidity has been noted in earlier studies, and the explanation for this effect has been discussed.[17] The rate constant for the DO^--catalyzed deuterium exchange reaction of the N-terminal α-amino proton **4-H_α^+** is 600-fold larger than that for the internal α-amino proton **4-H_α'** (Table 1). The presence

(38) Beak, P.; Siegel, B. *J. Am. Chem. Soc.* **1976**, *98*, 3601−3606.
(39) Streitwieser, A., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 755−759.
(40) Richard, J. P.; Amyes, T. L. *Curr. Opin. Chem. Biol.* **2001**, *5*, 626−633.
(41) Miller, B. G.; Snider, M. J.; Short, S. A.; Wolfenden, R. *Biochemistry* **2000**, *39*, 8113−8118.

of the terminal carboxylate anion at **4-H$_\alpha$′** is expected to result in only a small 4−5-fold decrease in k_{DO} for the deuterium exchange reaction of **4-H$_\alpha$′** as compared with that for an internal α-amino proton at a longer polyglycine peptide, for which the negative charge at the enolate is "shielded" from interaction with the C-terminal carboxylate anion.[42] Therefore, our data show that, neglecting the local effects of conformation and microenvironment, the isotope exchange and/or racemization reaction of a N-protonated N-terminal amino acid residue at a peptide or protein is ca. 130-fold faster than that of an identical internal amino acid residue. The more rapid deuterium exchange into N-terminal than into internal amino acid residues suggests that the determination of the extent of racemization of N-terminal amino acid residues would provide a measure of the aging of samples over a shorter time scale than is possible by monitoring the racemization of internal amino acid residues.[4−8]

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Supporting Information Available: Table S1: Rate constants k_{ex} (s^{-1}) for deuterium exchange into *N*-acetylglycine anion (**1**). Table S2: Rate constants k_{ex} (s^{-1}) for deuterium exchange into *N*-acetylglycinamide (**2**). Table S3: Rate constants k_{ex} (s^{-1}) for deuterium exchange into glycylglycine (**3**). Table S4: Rate constants k_{ex} (s^{-1}) for deuterium exchange into glycylglycylglycine (**4**) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(42) Neutralization of negative charge at **1** by converting the carboxylate anion to an amide to give **2** results in a 4.7-fold increase in k_{DO} for deuterium exchange into the acetyl methyl group (Table 1). The separation of the acetyl methyl group and the anionic C-terminal at **1** is similar to that of the internal α-CH_2 group and the anionic C-terminal at GlyGlyGly **4**. Therefore, we expect a similar 4−5-fold increase in k_{DO} to result from the shielding of internal α-CH_2 groups from the anionic C-terminal at polyglycine. This treatment neglects the much smaller effect on k_{DO} of shielding of the N-terminal α-CH_2 group from the anionic C-terminal at polyglycine.

(43) Gold, V.; Grist, S. *J. Chem. Soc., Perkin Trans. 2* **1972**, 89−95. Kresge, A. J.; O'Ferrall, R. A. M.; Powell, M. F. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: New York, 1987; Vol. 7.