Glycine Enolates: The Large Effect of Iminium Ion Formation on α-Amino Carbon Acidity

Ana Rios,[‡] Juan Crugeiras,[‡] Tina L. Amyes,[†] and John P. Richard*,[†]

Department of Chemistry, University at Buffalo SUNY, Buffalo New York 14260-3000
Departamento de Química Física, Facultad de Química Universidad de Santiago 15706 Santiago de Compostela, Spain

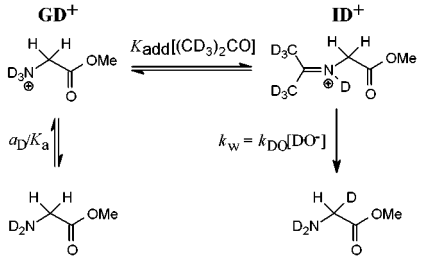
Received May 22, 2001
Revised Manuscript Received July 9, 2001

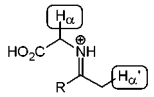
We wish to report extraordinarily efficient catalysis of deprotonation of the α-amino carbon of glycine methyl ester by the simple ketone acetone that is the result of a 10^7-fold larger acidity constant K_{CH} for carbon deprotonation of the iminium ion adduct $\mathbf{IH}^+$ ($pK_{CH} = 14$) than for deprotonation of *N*-protonated glycine methyl ester $\mathbf{GH}^+$ ($pK_{CH} = 21$).

The mechanism for enzyme-catalyzed deprotonation of carbon acids is a subject of some controversy and much interest.[1] The bulk of the rate acceleration for enzyme-catalyzed carbon deprotonation of α-amino acids is the result of stabilization of the amino acid enol(ate) relative to the very weak parent carbon acid.[1] We have shown that the acidity of the α-proton of glycine anion $H_2NCH_2CO_2^-$ is increased ca. 10^{12}-fold by the combined effects of *N*-protonation and *O*-methylation (a model for *O*-protonation) to give $^+H_3NCH_2CO_2Me$ ($\mathbf{GH}^+$).[2,3] It is well-known that formation of adducts of α-amino acids to the complex enzyme cofactor pyridoxal phosphate results in a large increase in the acidity of the α-amino carbon.[4] We now show that the carbon acidity of an α-amino acid ester is increased dramatically by formation of the iminium ion adduct to the simple ketone acetone.

Carbon deprotonation of glycine methyl ester, monitored by following exchange for deuterium of the first α-proton in D_2O, is second-order in the concentration of 3-quinuclidinone, in contrast to the first-order dependence observed for other general base catalysts such as 3-quinuclidinol.[2,3] This second-order term results from bifunctional catalysis by both the keto and amino groups of 3-quinuclidinone, since we have found that the simple ketone acetone is also a powerful catalyst of this deuterium exchange reaction. The observed rate constant for deuterium exchange into glycine methyl ester is increased by up to 1000-fold in the presence of acetone and phosphate buffer.[5] Amines, including α-amino acids,[6] provide very effective catalysis of deprotonation of aldehydes and ketones via the formation of iminium ion adducts (**1**, H_α').[6−9] Catalysis of deprotonation of α-amino acids through this adduct (**1**, H_α) might have been predicted, but not with such enormous catalytic power. In any event, there have been few reports of catalysis of carbon deprotonation of α-amino acids by ketones[10] and no studies of the mechanism of this reaction.

Scheme 1





1

The observed strong catalysis of deprotonation of the α-amino carbon of glycine methyl ester by acetone results from activation of the amino acid by formation of the iminium ion adduct $\mathbf{ID}^+$ (Scheme 1). The equilibrium constant $K_{add} = 0.0033$ M^{-1} for formation of $\mathbf{ID}^+$ from *N*-protonated glycine methyl ester $\mathbf{GD}^+$ and d_6-acetone in D_2O at 25 °C and $I = 1.0$ (KCl) was determined by monitoring the formation of small amounts of $\mathbf{ID}^+$ by ^1H NMR spectroscopy (Scheme 1).[11,12] The apparent acidity constant for glycine methyl ester in D_2O at 25 °C and $I = 1.0$ (KCl) was determined by NMR titration as $K_a = 3.2 \times 10^{-9}$ M and is in good agreement with our earlier potentiometric value.[3]

The exchange for deuterium of the first α-proton of glycine methyl ester in D_2O at 25 °C and $I = 1.0$ (KCl) was followed by ^1H NMR spectroscopy at 500 MHz.[2,3,11,13,14] Table S1 of the Supporting Information gives the observed first-order rate constants k_{ex} (s^{-1}) for deuterium exchange in the presence of various concentrations of acetone and buffer catalysts at pD 7.64 and 6.61 (phosphate buffer) and at pD 5.56 (acetate buffer) that were determined by published procedures.[15] Figure 1A shows the linear dependence of k_{ex} (s^{-1}) on the total concentration of phosphate buffer (pD 7.64) in the presence of different fixed concentrations of acetone. The slopes of these correlations are the second-order rate constants $(k_B)_{obsd}$ (M^{-1} s^{-1}) for deuterium exchange into glycine methyl ester catalyzed by phosphate buffer at the given concentration of acetone. These data will be discussed in a full

* To whom correspondence should be addressed. Telephone: 716-645-6800, ext 2194. Fax: 716-645-6963. Email: jrichard@chem.buffalo.edu.
† University at Buffalo, SUNY.
‡ Universidad de Santiago.

(1) Gerlt, J. A.; Kozarich, J. W.; Kenyon, G. L.; Gassman, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9667−9669. Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 11552−11568. Guthrie, J. P.; Kluger, R. *J. Am. Chem. Soc.* **1993**, *115*, 11569−11572.
(2) Rios, A.; Richard, J. P. *J. Am. Chem. Soc.* **1997**, *119*, 8375−8376.
(3) Rios, A.; Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **2000**, *122*, 9373−9385.
(4) Martell, A. E. *Acc. Chem. Res.* **1989**, *22*, 115−124.
(5) For example, the estimated first-order rate constant for deprotonation of glycine methyl ester in the presence of 1.0 M acetone and 1.0 M phosphate buffer at pD 6.61 is 1000-fold larger than the rate constant for the reaction in the absence of these catalysts.
(6) Hine, J.; Menon, B. C.; Mulders, J.; Flachskam, R. L. *J. Org. Chem.* **1969**, *34*, 4083−4087.
(7) Bender, M. L.; Williams, A. *J. Am. Chem. Soc.* **1966**, *88*, 2502−2508.
(8) Hine, J.; Kokesh, F. C.; Hampton, K. G.; Mulders, J. *J. Am. Chem. Soc.* **1967**, *89*, 1205−1211.
(9) Hupe, D. J.; Kendall, M. C. R.; Spencer, T. A. *J. Am. Chem. Soc.* **1973**, *95*, 2271−2278.
(10) Barry, L. G.; Pugniere, M.; Castro, B.; Previero, A. *Int. J. Pept. Protein Res.* **1993**, *41*, 323−325. Parmar, V. S.; Singh, A.; Bish, K. S.; Kumar, N.; Belokon, Y. N.; Kochetkov, K. A.; Ikonnikov, N. S.; Orlova, S. A.; Tararov, V. I.; Saveleva, T. F. *J. Org. Chem.* **1996**, *61*, 1223−1227.
(11) ^1H NMR spectra at 500 MHz were recorded in D_2O on a Varian Unity Inova 500 NMR spectrometer or a Bruker AMX 500 NMR spectrometer using the operating conditions described in earlier work.[2,3]
(12) K_{add} was determined as the slope of a plot of the ratio of the integrated peak areas for the CH_2 groups of $\mathbf{ID}^+$ (4.73 ppm) and $\mathbf{GD}^+$ (3.97 ppm) against the concentration of acetone at pD = 4.5, where both species are present exclusively in their *N*-protonated cationic forms.
(13) Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **1992**, *114*, 10297−10302.
(14) Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **1996**, *118*, 3129−3141.
(15) Values of k_{ex} for reactions at pD 5.56 and 6.61 were determined by monitoring the formation of monodeuterated glycine methyl ester by ^1H NMR.[3] Values of k_{ex} for reactions at pD 7.64 were determined from the observed rate constant for hydrolysis of glycine methyl ester, k_{hyd} (s^{-1}), and the ratio of the concentrations of fully protonated [gly-H] and monodeuterated [gly-D] glycine products determined by ^1H NMR analysis after complete hydrolysis of the ester, according to eq 5. $k_{ex} = k_{hyd}/(\{[\text{gly-H}]/[\text{gly-D}]\} - 0.5)$ (5).

10.1021/ja016250c CCC: $20.00
Published on Web 07/20/2001

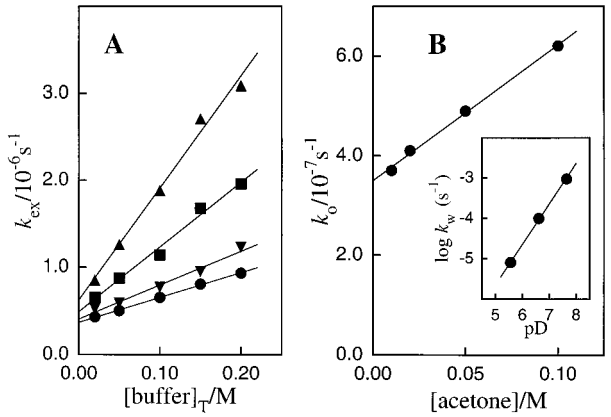


Figure 1. (A) Dependence of k_{ex} (s^{-1}) for exchange for deuterium of the first α-proton of glycine methyl ester in the presence of acetone on the total concentration of phosphate buffer (pD 7.64) in D_2O at 25 °C and $I = 1.0$ (KCl). Key: (●) 0.01 M acetone; (▼) 0.02 M; (■) 0.05 M; (▲) 0.10 M. (B) Dependence of k_o (s^{-1}), determined as the intercepts of the correlations in Figure 1A, on [acetone]. The slope gives $(k_w)_{obsd}$ (M^{-1} s^{-1}) for acetone-catalyzed deuterium exchange into glycine methyl ester at pD 7.64. Inset: pD-rate profile for deprotonation of $\mathbf{ID}^+$. Values of k_w were determined from $(k_w)_{obsd}$ using eq 1 (see text). The solid line of unit slope shows the fit of the data to eq 2 which gives $k_{DO} = 13\,000$ M^{-1} s^{-1} for deprotonation of $\mathbf{ID}^+$ by DO^-.

report. The intercepts are the first-order rate constants $k_o = k_w f_{ID}$ (s^{-1}) for deuterium exchange at the given concentration of acetone, where $f_{ID} = K_{add}[\text{acetone}]/\{1 + K_a/a_D\}$ ($a_D = 10^{-pD}$) is the fraction of glycine methyl ester present as the iminium ion adduct $\mathbf{ID}^+$,[16] and $k_w = k_{DO}[DO^-]$ (s^{-1}) is the first-order rate constant for deprotonation of $\mathbf{ID}^+$ by deuterioxide ion (Scheme 1).

Figure 1B shows the dependence of the values of k_o (s^{-1}) from Figure 1A on the concentration of acetone. The slope of this correlation is the observed second-order rate constant for acetone-catalyzed deprotonation of glycine methyl ester by deuterioxide ion at pD 7.64, $(k_w)_{obsd} = 2.7 \times 10^{-6}$ M^{-1} s^{-1} (eq 1). This was substituted into eq 1 with $K_{add} = 0.0033$ M^{-1}, $K_a = 3.2 \times 10^{-9}$ M and $a_D = 10^{-7.64}$ M to give $k_w = 9.3 \times 10^{-4}$ s^{-1} for deprotonation of $\mathbf{ID}^+$ by deuterioxide ion at pD 7.64.

$$(k_w)_{obsd} = \frac{k_w K_{add}}{\left(1 + \frac{K_a}{a_D}\right)} \quad (1)$$

$$\log k_w = \log\left(\frac{k_{DO} K_w}{\gamma_{OL}}\right) + pD \quad (2)$$

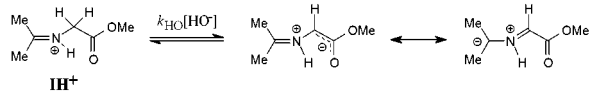
$$\log k_{HO} = 10.2 - 0.44 pK_{CH} \quad (3)$$

$$pK_{CH} = \left(\frac{10.2 - \log k_{HO}}{0.44}\right) \quad (4)$$

The same treatment of the data for reactions at pD 6.61 and 5.56 (Table S1) gives $k_w = 1.0 \times 10^{-4}$ M^{-1} s^{-1} and $k_w = 8.2 \times 10^{-6}$ M^{-1} s^{-1}, respectively. The inset in Figure 1B shows the linear logarithmic plot of the values of k_w against pD. The solid line of unit slope shows the fit of the data to eq 2, where $K_w = 10^{-14.87}$ M^2 is the ionization constant of D_2O at 25 °C,[17] and $\gamma_{OL} = 0.79$ is the apparent activity coefficient of lyoxide ion

(16) We make the assumption that the concentration of the neutral imine adduct is negligible because the equilibrium constant K_{add} for formation of the imine/iminium ion is very small.

Scheme 2



determined under our reaction conditions.[14] The data give $k_{DO} = 13{,}000$ M^{-1} s^{-1} as the second-order rate constant for deprotonation of the iminium ion $\mathbf{ID}^+$ by deuterioxide ion. This can be combined with an estimated solvent deuterium isotope effect of $k_{DO}/k_{HO} = 1.46$ to give $k_{HO} = 9000$ M^{-1} s^{-1} for deprotonation of $\mathbf{IH}^+$ by hydroxide ion in H_2O (Scheme 2).[18]

Equations 3 and 4 describe the observed linear logarithmic correlation between k_{HO} and the carbon acidity pK_{CH} of cationic ketones and esters.[19] Substitution of $k_{HO} = 9000$ M^{-1} s^{-1} for deprotonation of the iminium ion $\mathbf{IH}^+$ into eq 4 gives an estimated value of $pK_{CH} = 14$ for deprotonation of the α-imino carbon of $\mathbf{IH}^+$ to form the enolate zwitterion (Scheme 2). This is 7 p*K* units lower than $pK_{CH} = 21.0$ for deprotonation of $\mathbf{GH}^+$ at the α-amino carbon.[2,3] Therefore, a modest chemical modification of the amino acid glycine results in a very substantial movement of the pK_a of the α-protons toward physiological pH. The formation of iminium ion adducts between α-amino acids and the enzyme cofactor pyridoxal phosphate also results in a large increase in the acidity of the α-protons.[21] Our data show that a large fraction of the effect of this cofactor on carbon acidity is also observed for the much simpler iminium ion $\mathbf{IH}^+$.

We propose that the large 7 p*K* unit effect of iminium ion formation on the carbon acidity of glycine methyl ester represents the additivity of two smaller effects: (1) The stabilization of the enolate by direct delocalization of negative charge onto the α-imino group (Scheme 2). A similar delocalization of charge results in a ca. 3-unit lower pK_a of 15.2 for the C-2 proton of 3-cyclohexenone[22] compared with the pK_a of 18.1 for cyclohexanone.[20a] (2) The enhancement of intramolecular electrostatic stabilization of the enolate anion by interaction with the cationic nitrogen when the amino protons of $\mathbf{GH}^+$ are replaced by an organic fragment to give $\mathbf{IH}^+$.[3] This results in a 3-unit larger acidifying effect of the α-NMe_3^+ group at betaine methyl ester ($pK_{CH} = 18.0$) than of the α-NH_3^+ group at $\mathbf{GH}^+$ ($pK_{CH} = 21.0$).[3] The large intramolecular electrostatic stabilization of zwitterionic enolates has been thoroughly documented in earlier work.[23]

Acknowledgment. We acknowledge National Institutes of Health Grant GM 39754 for generous support, and a grant to A.R. from the Dirección General de Investigación Científica y Enseñanza Superior.

Supporting Information Available: Table S1: rate constants k_{ex} (s^{-1}) for deuterium exchange into glycine methyl ester in the presence of acetone and buffer catalysts (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA016250C

(17) Covington, A. K.; Robinson, R. A.; Bates, R. G. *J. Phys. Chem.* **1966**, *70*, 3820−3824.

(18) This is the secondary solvent deuterium isotope effect determined for deprotonation of acetone by hydroxide ion at 25 °C: Pocker, Y. *Chem. Ind.* **1959**, 1383−1384.

(19) This correlation was obtained using the following rate and equilibrium constants: (i) $C_6H_5N^+CH_2C(O)Ph$: $k_{HO} = 1.8 \times 10^5$ M^{-1} s^{-1}; $pK_{CH} = 10.9$.[20] (ii) Betaine methyl ester: $k_{HO} = 390$ M^{-1} s^{-1}; $pK_{CH} = 18.0$.[3] (iii) *N*-protonated glycine methyl ester: $k_{HO} = 4.1$ M^{-1} s^{-1}; $pK_{CH} = 21.0$.[3]

(20) (a) Keeffe, J. R.; Kresge, A. J. In *The Chemistry of Enols*; Rappoport, Z., Ed.; John Wiley and Sons: Chichester, 1990; pp 399−480. (b) Carey, A. R. E.; Al-Quatami, S.; More O'Ferrall, R. A.; Murray, B. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1097−1098.

(21) A less thoroughly documented pK_a of 9 has been estimated for the α-proton of the iminium ion adduct of alanine with 3-hydroxypyridine-4-carboxaldehyde: Dixon, J. E.; Bruice, T. C. *Biochemistry* **1973**, *12*, 4762−4766.

(22) Dzingeleski, G. D.; Blotny, G.; Pollack, R. M. *J. Org. Chem.* **1990**, *55*, 1019−1023.

(23) Tobin, J. B.; Frey, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 12253−12260.