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Are Carboxyl Groups the Most Acidic Sites in Amino Acids? Gas-Phase Acidity, H/D Exchange Experiments, and Computations on Cysteine and Its Conjugate Base

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Abstract: Hydrogen–deuterium exchange experiments were carried out on the conjugate base of cysteine with four different deuterated alcohols. Three H/D exchanges are observed to take place in each case, and a relay mechanism which requires the SH and CO₂H groups to have similar acidities and subsequently proceeds through a zwitterionic intermediate is proposed. Gas-phase acidity measurements also were carried out in a quadrupole ion trap using the extended kinetic method and in a Fourier transform mass spectrometer by an equilibrium determination. The results are in excellent accord with each other and high-level ab initio and density functional theory calculations and indicate that the side-chain thiol in cysteine is more acidic than the carboxyl group by 3.1 kcal mol^{−1}. Deprotonated cysteine is thus predicted to be a thiolate ion. A zwitterionic species also was located on the potential energy surface, but it is energetically unfavorable (+10.1 kcal mol^{−1}).

Introduction

Relative gas-phase acidities for 18 of the 20 essential amino acids were measured by the original (standard) variant of the Cooks kinetic method in which entropic effects are assumed to cancel.^{1,2} Absolute values were reported since equilibrium acidity measurements are available for glycine and alanine.³ The results span a relatively narrow range from 331 to 342 kcal mol^{−1}, and since none of the side-chain substituents are more acidic than a carboxylic acid, it is widely accepted that the conjugate bases of amino acids are carboxylate ions (RCO₂[−]). This is a seemingly reasonable conclusion, but the presence of two or more functional groups in a compound can significantly alter its physical and thermodynamic properties. For example, it is well-known that benzoic acid is a stronger acid than phenol by 10 kcal mol^{−1} (i.e., $\Delta H^0_{\text{acid}} = 340.2 \pm 2.2$ vs 350.4 ± 0.6 kcal mol^{−1}),⁴ but the hydroxyl (OH) group is more acidic than the carboxyl (CO₂H) position in *p*-hydroxybenzoic acid (*p*-HOC₆H₄CO₂H) because the latter substituent stabilizes the former one more than the other way around.⁵ As a consequence, this raises the question: are the carboxyl groups really the most

acidic sites in amino acids? We report herein the results of gas-phase acidity and H/D exchange measurements on cysteine and its conjugate base along with high-level computations. The thiol group is found to be the most acidic site in cysteine, and its conjugate base therefore is a thiolate and not a carboxylate ion.

Experimental Section

Benzyl Alcohol (1.1 equiv)–OD. A 60% suspension of sodium hydride in mineral oil was washed with dry pentane under an argon atmosphere, and the residual solvent was removed under vacuum. Undiluted benzyl alcohol was added to the reaction flask slowly, and the resulting suspension was stirred for 1 h at room temperature before being quenched with 2 equiv of D₂O (99.9% atom %D). The deuterated alcohol was extracted with dry ether, and the organic layer was dried over sodium sulfate before removing the solvent under vacuum with a mechanical pump to afford C₆H₅CH₂OD (>96 atom %D by ¹H NMR). All other chemicals used in this work were obtained commercially and used as supplied.

Gas-phase H/D exchange of deprotonated cysteine with EtOD (99.5+ atom %D), CF₃CH₂OD (99 atom %D), C₆H₅CH₂OD (96+ atom %D), and C₆D₅OD (99+ atom %D) were carried out in a dual-cell model 2001 Finnigan Fourier transform mass spectrometer (FTMS) equipped with a 3 T superconducting magnet and controlled by a Sun workstation running the Odyssey version 4.2 software package or a single cylindrical cell 3 T ESI instrument supplied by IonSpec Co. In the former case, cysteine was added into the source cell via a heated solid inlet probe and its *M* − 1 ion (*d*₀) was produced by electron ionization (2.5 eV). After equilibrating with the neutral acid, all of the ions were transferred to the analyzer cell and the *d*₀ ion (*m/z* 120) was isolated using a stored-waveform inverse Fourier transform (SWIFT) excitation.⁶ In the latter

[†] The College of William and Mary.

[‡] University of Minnesota.

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instrument, a 200 μM solution of cysteine dissolved in a 3:1 (v/v) mixture of methanol and water with enough lithium hydroxide to make the solution slightly basic was pumped at a flow rate of 10 $\mu\text{L}/\text{min}$ into a Z-spray (Micromass) ESI source. The extracted ions were accumulated in a hexapole to build up signal and then were injected into a radio frequency (rf) only quadrupole ion guide and transported into the FTMS cell, where they were cooled by a pulse of argon and trapped by the dynamically assisted gated trapping technique.⁷ The $M - 1$ ion of cysteine was isolated using an arbitrary waveform excitation, and its reactivity was effectively the same regardless of which instrument was used.

Hydrogen–deuterium exchange reactions were monitored as a function of time with four different deuterated reagents which were leaked into the system at pressures between 1×10^{-7} and 5×10^{-7} torr for a minimum of 5 h immediately prior to carrying out experiments. The effective deuterium content of the deuterated reagents was determined by examining the H/D exchange of isophthalate ($m\text{-C}_6\text{H}_4(\text{CO}_2\text{H})\text{CO}_2^-$), since it reacts at the collision-controlled rate with all of the deuterated alcohols used in this work and incorporates only one deuterium atom. As a result, the final d_1/d_0 ratio provides the useful deuterium content of the exchange reagent in the reaction region.⁸ This was found to be 90–100 %D in each case. Pseudo-first-order bimolecular H/D exchange reaction rate constants were determined from the decay of the reactant (d_0) ion and the pressure of the alcohol in the reaction region; no correction was made for the small amount of protic material. In all cases, the linear fit of the experimental data gave $r^2 \geq 0.998$. Apparent and site-specific rate constants also were computed using a kinetic program developed by He and Marshall,⁹ and in this case the deuterium content of the reagent and reverse processes (e.g., $d_1 \rightarrow d_0$) were accounted for in the rate determination. As apparent and site-specific rate constants give essentially the same information when the former is statistically corrected, only the latter rates are presented herein, but all of the rate constants, and graphical examples of the data fits, are given in the Supporting Information.

An equilibrium acidity measurement was carried out on cysteine in the dual-cell instrument by measuring forward and reverse proton-transfer rate constants with chloroacetic acid. In one direction, the amount of cysteine added to the cell needs to be determined. This is difficult because it is not very volatile and was added via the solid probe inlet. As a result, the sample is added ~ 1 cm from the reaction region and ~ 1 m from the ionization gauge used to measure its pressure. This leads to a pressure differential which must be addressed. It was dealt with by measuring the reaction rates of cysteine with hydroxide and fluoride ions one after the other since these reactions safely can be assumed to occur at the collision limit.¹⁰ The resulting pressure correction factors were 7.45 and 7.32, and the average of these two values was employed. The B3LYP/aug-cc-pvdz dipole moment for cysteine of 4.58 D was used in computing average dipole orientation (ADO) rates,¹¹ but the resulting acidity is not very sensitive to this value (i.e., dipole moments spanning from 4.0–5.0 D alter ΔH_{acid}^0 by only up to 0.1 kcal mol⁻¹).

An electrospray ionization–quadrupole ion trap instrument was used to carry out kinetic acidity measurements via procedures that have been given in detail elsewhere.^{12–14} Briefly, dilute ($\sim 5\text{--}10 \times 10^{-4}$ M) solutions of cysteine and one of a series of reference acids in slightly

basic (1% NH_4OH) 49.5:49.5 $\text{H}_2\text{O}/\text{MeOH}$ were directly infused at flow rates of 10–20 $\mu\text{L}/\text{min}$ into an LCQ DECA ion trap mass spectrometer. Solution and ion-focusing conditions were adjusted in order to maximize the formation of proton-bound dimer ions of the form $[\text{A}^- - \text{H}^+ - \text{B}_i^-]^-$, where A^- is deprotonated cysteine and B_i^- is the deprotonated reference acid. The proton-bound dimer ions were isolated at $q_z = 0.250$ V with a mass-width adjusted to maximize ion signal while still maintaining isolation. The isolated ions were allowed to undergo collision-induced dissociation with the background helium atoms. The ratio of the deprotonated reference acid to deprotonated cysteine was obtained by performing an activation amplitude scan from 0% to 100% in steps of 2. The final ion ratios are averages of at least three scans obtained on several different days.

Gas-phase acidities and entropy contributions were obtained from the extended kinetic method that has been described in detail elsewhere.^{15–18} The final version of the extended kinetic method takes the form

$$\ln\left(\frac{I_{\text{B}_i}}{I_{\text{A}^-}}\right) \approx \frac{\Delta H_{\text{B}_i}^0 - \Delta H_{\text{av}}^0}{RT_{\text{eff}}} - \frac{\Delta H_{\text{A}^-}^0 - \Delta H_{\text{av}}^0}{RT_{\text{eff}}} + \frac{\Delta S_{\text{B}_i}^0}{R} - \frac{\Delta S_{\text{A}^-}^0}{R}$$

A plot of $\ln[I(\text{B}_i^-)/I(\text{A}^-)]$ versus $\Delta H_{\text{B}_i}^0 - \Delta H_{\text{av}}^0$ is generated, where $\Delta H_{\text{B}_i}^0$ is the gas-phase acidity of reference acid i and ΔH_{av}^0 is the average acidity of the set of reference acids. This procedure is repeated for data obtained at each of the collision energies. Rather than using the traditional form of the extended kinetic method in which a second plot is generated, the orthogonal distance regression (ODR) method of Ervin and Armentrout was used to extract the gas-phase acidity and entropy contribution.¹⁹ This approach has been shown to give more realistic uncertainties for the derived thermochemical values. The ODR procedure uses ion ratios from n reference acids and m different collision energies and is used to create m best-fit lines to the data, forcing them to cross at a single isothermal point. The x -coordinate of the isothermal point is $\Delta H_{\text{A}^-}^0 - \Delta H_{\text{av}}^0$, and the y -coordinate is $\Delta S_{\text{A}^-}^0/R$, where $\Delta H_{\text{A}^-}^0$ and $\Delta S_{\text{A}^-}^0$ are the gas-phase acidity and deprotonation entropy for cysteine, respectively. Final uncertainties are obtained from Monte Carlo simulations in which random noise is generated within user-defined ranges of the uncertainties in the gas-phase acidity of each reference acid and in the experimental ion ratios. For these studies, the uncertainty in the reference acids was taken to be ± 2 kcal mol⁻¹ and the uncertainty in the $\ln(\text{ratio})$ values was ± 0.05 .

Computational Methods. Geometry optimizations were carried out on cysteine and its conjugate base using the Becke three-parameter hybrid exchange and Lee–Yang–Parr correlation density functional (i.e., B3LYP)²⁰ and Dunning’s augmented correlation-consistent double- ζ basis set (i.e., aug-cc-pvdz)²¹ on workstations at the Minnesota Supercomputer Institute running Gaussian 03.²² Both thiolate and carboxylate ions were examined, and a variety of conformations were probed. In an attempt to ensure that the most stable structures were located, Monte Carlo calculations using the MMFF force field were run using Spartan 04 on a Macintosh PowerPC G4 computer.²³ The 10 most stable species for cysteine and its $M - 1$ ion were reoptimized using density functional theory. Vibrational frequencies subsequently were computed, and unscaled values were used to provide zero-point energies (ZPEs) and thermal corrections to 298 K. G3B3 calculations,²⁴

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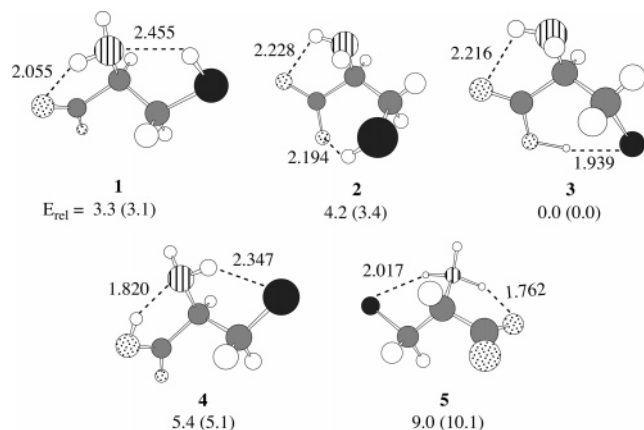


Figure 1. B3LYP/aug-cc-pvdz optimized structures and relative energies at 298 K (kcal mol⁻¹) for the conjugate base of cysteine; parenthetical values are for G3B3 results.

a variant of G3 theory,²⁵ also were performed as described in the literature, and all of the resulting energetic quantities reported herein are given at 298 K.

Independent calculations were performed at the College of William and Mary. The GMMX conformational searching routine in PCModel was used to generate low-energy structures for cysteine and its carboxylate anion.²⁶ Structures for the thiolate ion were generated from the carboxylates and cysteine structures located from the GMMX search by moving or removing a proton. The 30 lowest energy species for cysteine, its carboxylate, and its thiolate anion were subjected to varying levels of density functional theory, with geometries ultimately optimized at the B3LYP/6-31+G(d) level. Single-point energies were obtained at the B3LYP/6-311++G(d,p) level of the theory. Zero-point energy and thermal corrections were obtained in the standard manner from unscaled B3LYP/6-31+G(d) vibrational frequencies. Predictions for the gas-phase acidity of the different acidic sites of cysteine were obtained using an isodesmic approach with acetic acid serving as the reference acid.

Results and Discussion

Calculations. Cysteine and its conjugate base were fully optimized at the B3LYP/aug-cc-pvdz level. Several selected carboxylate and thiolate anion structures were examined, and then Monte Carlo searches using the MMFF force field were carried out to look for additional conformations. In the latter case, the lowest energy leads for both the carboxylate and thiolate were reoptimized using various levels of density functional theory. Ultimately, low-energy conformers were optimized at both the B3LYP/aug-cc-pvdz and B3LYP/6-31+G(d) levels, and the lowest energy carboxylates (**1** and **2**), thiolates (**3** and **4**), and zwitterion (**5**) are illustrated in Figure 1.²⁷ Structures obtained from the two different basis sets are essentially identical in their hydrogen-bonding configurations. Each structure has two hydrogen bonds, and the most favorable carboxylate (**1**) has CO₂⁻⋯HN and N⋯HS interactions, whereas they are between S⁻⋯HO₂C and C=O⋯HN in the lowest energy thiolate (**3**). In the former case, the thiol–amine interaction must make the amine a better hydrogen-bond donor than usual otherwise **2** would be expected to be more stable than **1** since its CO₂⁻⋯HS hydrogen bond involves the matching of two groups (i.e., RCO₂H and RSH) with similar acidities.

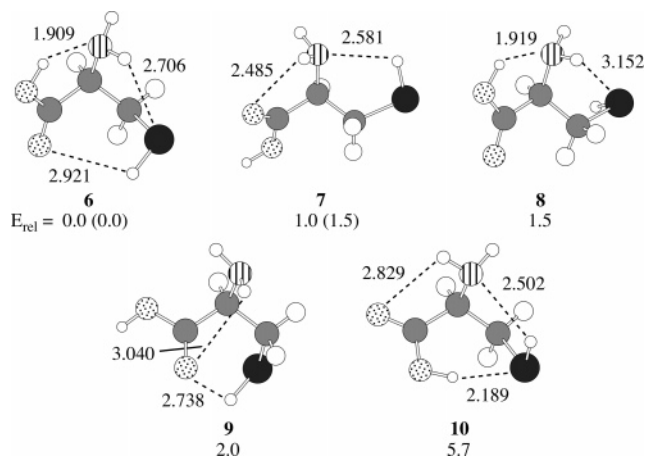


Figure 2. B3LYP/aug-cc-pvdz optimized structures and relative energies at 298 K (kcal mol⁻¹) for cysteine; parenthetical values are for G3B3 results.

The conformations of neutral cysteine were explored in a similar manner, and the lowest five energy structures (**6**–**10**) are given in Figure 2. The most stable species (**6**) is the same as recently reported by Bleiholder et al.²⁸ and has three intramolecular hydrogen bonds; one between the carboxyl group and the amine (CO₂H⋯N), the second between the amine and the thiol (NH⋯S), and the third between the thiol and the carbonyl of the carboxyl group (SH⋯O=C). All of the other structures only have two hydrogen bonds.

Thiolate anion **3** is predicted to be 3.3 kcal mol⁻¹ more stable than carboxylate **1** at 298 K (Figure 1) at the B3LYP/aug-cc-pvdz level and 2.0 kcal/mol more stable at the B3LYP/6-311++G(d,p)//B3LYP/6-31+G(d) level. This result is unexpected in that carboxylic acids are more acidic than thiols (i.e., $\Delta\Delta H^0_{\text{acid}}$ (CH₃CH₂CH₂SH – CH₃CH₂CO₂H) = 6.8 kcal mol⁻¹),²⁹ and it indicates that the most acidic position in cysteine is at the sulfur *not* the oxygen of the carboxyl group. In water the opposite is observed, and the pK_a of the carboxyl group (1.7) is 6.6 pK_a units smaller or 9.0 kcal mol⁻¹ more acidic than the thiol (8.3).³⁰ This suggests that in the gas phase intramolecular hydrogen bonding in cysteine overcomes the inherent preference for oxygen deprotonation, and in solution this effect is diminished. However, the difference in energy between **1** and **3** is only 3 kcal mol⁻¹, which is about the anticipated accuracy of the computations, so their relative stabilities could be reversed. To address this possibility, high-level G3B3 computations were carried out on **1**–**5**, and there is little change in their relative stabilities. Thiolate **3** is still predicted to be more stable than carboxylate **1** ($\Delta\Delta H^0 = 3.1$ kcal mol⁻¹), which leads to the conclusion that the thermodynamic deprotonation of cysteine affords a sulfur anion. To the best of our knowledge this represents the first suggestion that the thermodynamic deprotonation of an amino acid affords anything but a carboxylate ion.³¹ A zwitterionic structure

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(26) PCModel, version 8.5; Serena Software: Bloomington, IN, 2003.

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(29) The experimental acidities are 354.2 ± 2.2 and 347.4 ± 2.2 kcal mol⁻¹ (see ref 4), respectively, and are well reproduced by B3LYP/aug-cc-pvdz (352.5 and 345.1 kcal mol⁻¹) and G3B3 (355.0 and 347.7 kcal mol⁻¹) computations.

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Table 1. Apparent Rate Constants for the H/D Exchange Reactions of the Cysteine M – 1 Ion with Deuterated Alcohols

ROD	ΔH_{acid}^0 ^a	$k \times 10^{12} \text{ (cm}^3 \text{ molecule}^{-1} \text{ s}^{-1})^{b,c}$		
		$d_0 \rightarrow d_1$	$d_1 \rightarrow d_2$	$d_2 \rightarrow d_3$
CH ₃ CH ₂ OD	378.3 ± 1.0	1.4 ± 0.4	0.78 ± 0.23 (1.2 ± 0.4)	<i>d</i>
C ₆ H ₅ CH ₂ OD	370.0 ± 2.1	31 ± 9	19 ± 6 (29 ± 9)	8.1 ± 2.4 (24 ± 7)
CF ₃ CH ₂ OD	361.7 ± 2.2	280 ± 80	220 ± 70 (330 ± 110)	110 ± 30 (330 ± 90)
C ₆ D ₅ OD	350.4 ± 0.6	1070 ± 320	770 ± 230 (1160 ± 350)	280 ± 80 (840 ± 240)

^a All values are for the protic acids and come from ref 4. ^b The uncertainties were assumed to be ±30%. ^c Parenthetical values are statistically corrected assuming all three hydrogens are kinetically equivalent. That is, the values for $d_1 \rightarrow d_2$ and $d_2 \rightarrow d_3$ were multiplied by 1.5 and 3, respectively. ^d This reaction was too slow for us to accurately measure its rate.

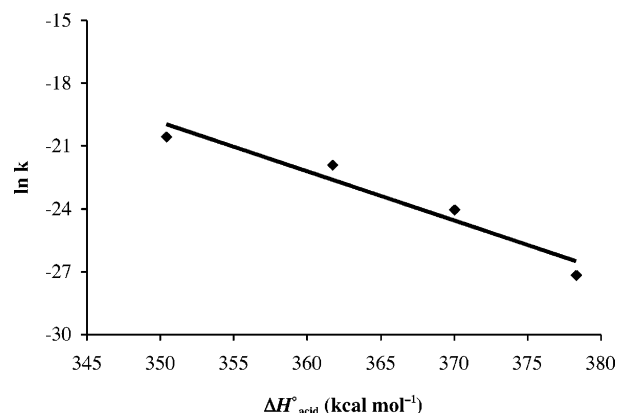
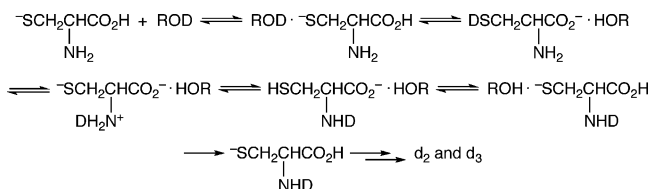
containing a thiolate, a carboxylate, and an ammonium ion (5) also is worth noting, but it is not energetically competitive with 1–4 and will not contribute significantly to the structure of the cysteine M – 1 ion.

Our calculations lead to a predicted acidity of 331.4 (B3LYP/6-311++G(d,p)//B3LYP/6-31+G(d)), 332.3 (B3LYP/aug-cc-pvdz), and 333.3 (G3B3) kcal mol^{–1} for cysteine and a small 3 kcal mol^{–1} energetic difference between deprotonation at the carboxyl and thiol groups.³² To experimentally test these predictions, the H/D exchange behavior of the conjugate base of cysteine was examined, the acidity of this amino acid was reinvestigated using the extended kinetic method, and an equilibrium determination was carried out.

Hydrogen–Deuterium Exchange. Deprotonated cysteine undergoes three hydrogen–deuterium (H/D) exchanges with ethanol–OD, benzyl alcohol–OD, 2,2,2-trifluoroethanol–OD, and phenol–OD with apparent rate constants that span from 10^{–12} to 10^{–9} cm³ molecule^{–1} s^{–1} (Table 1). These reactions range from quite slow and inefficient (i.e., ~1 in a 1000 collisions) to fast and very efficient (i.e., collision controlled) and are directly correlated with the acidity of the exchange reagent (Figure 3). Upon statistically correcting the rate coefficients for the number of exchangeable hydrogens, all of the H/D exchanges occur at the same rate within the experimental uncertainty. That is, the amino hydrogens and the carboxyl hydrogen are replaced by deuterium with one site-specific rate constant.³³ This indicates that the H/D exchange of these two groups is kinetically related and occurs via one pathway. In the reaction with EtOD, the exchange mechanism cannot involve a proton-transfer step leading to cysteine in its neutral or zwitterionic form and ethoxide because ethanol is 45 kcal mol^{–1} less acidic than the G3B3 acidity of cysteine and there simply is not enough energy in the system to fuel such an endothermic process. The same situation applies to benzyl alcohol and probably 2,2,2-trifluoroethanol, which suggests that one common mechanism involving a zwitterion intermediate and several relay processes³⁴ is operating for all four exchange reagents used in this study. Such a mechanism which accounts for all of the data

(32) The B3LYP acidities were corrected for the computed error in propionic (aug-cc-pvdz) or acetic (6-31++G(d,p)) acid (i.e., isodesmic reactions were used). To obtain the directly calculated acidities subtract 2.3 from the values in the text. The analogous correction would be to subtract 0.3 kcal mol^{–1} from the G3B3 acidity, but this was not done, and the directly computed value is given.

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**Figure 3.** Hydrogen exchange rates of the cysteine M – 1 ion ($d_0 \rightarrow d_1$) vs gas-phase acidities. The equation for the least-squares line is $\ln k = -0.236 \Delta H_{\text{acid}}^0 + 62.6$, $r^2 = 0.94$.**Scheme 1**

and is consistent with the computational modeling that we have done is given in Scheme 1. The key processes involve the conversion of the initial thiolate to a carboxylate via a relay process followed by the formation of a zwitterionic anion intermediate. A subsequent reversal of the steps leads to the incorporation of three deuterium atoms with one site-specific rate constant. Alternatively, the zwitterionic intermediate could revert to the thiolate directly, but this pathway is predicted to be a little less favorable on the basis of our computations (see the Supporting Information for additional details). In either case, this mechanism accounts for the observed three H/D exchanges, explains why the carboxyl and amino groups react at the same rate, and provides a rationale for why deuterated reagents which are much less acidic than cysteine induce isotopic exchange. It also requires that the thiolate and carboxylate anions be similar in energy as predicted by computations.

Acidity. The gas-phase acidity of cysteine was determined using the extended kinetic method in an ESI–quadrupole ion trap instrument.^{15–18} The following reference acids and their acidities in kcal mol^{–1} were used: 2,2-dimethyl-1,3-dioxane-4,6-dione ($\Delta H_{\text{acid}}^0 = 331.9 \pm 0.1$), 3-(trifluoromethyl)benzoic acid ($\Delta H_{\text{acid}}^0 = 332.2 \pm 2.1$), 4'-hydroxyacetophenone ($\Delta H_{\text{acid}}^0 = 335.5 \pm 2.1$), 3-nitrophenol ($\Delta H_{\text{acid}}^0 = 334.4 \pm 2.1$), and 4-chlorobenzoic acid ($\Delta H_{\text{acid}}^0 = 335.5 \pm 2.1$).⁴ A plot of $\ln(A^-/B_i^-)$ versus $\Delta H_{\text{acid}}^0(B_i) - \Delta H_{\text{acid,av}}^0$ is shown in Figure 4. The experiment was repeated at activation amplitudes between 0% and 100% (0–5 V lab). For activation amplitudes above 52% the effective temperature leveled off and was not included in the final acidity determination. The ODR-derived lines are also shown in Figure 4. The isothermal point leads to a ΔH_{acid}^0 of 332.9 ± 3.3 kcal mol^{–1} where the uncertainty is at the 95% confidence level as obtained from the Monte Carlo simulations in the ODR procedure. In addition, an entropy of deprotonation

(34) For a description of different hydrogen–deuterium exchange pathways, see: Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 12840–12854.

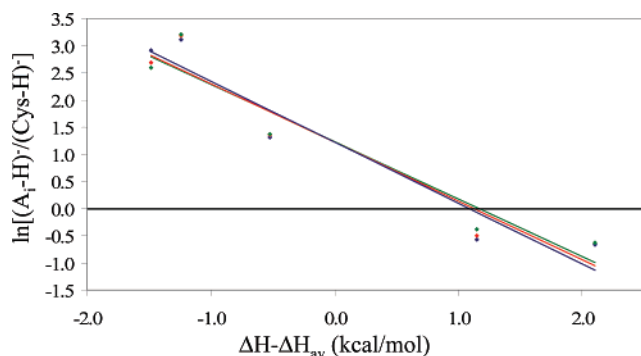
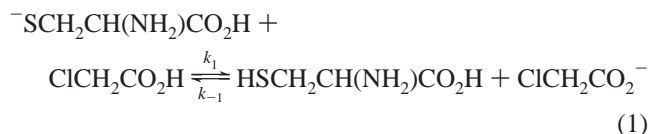


Figure 4. Plot of $\ln[B_i^-/A^-]$ vs $\Delta H^0 - \Delta H^0_{av}$. Data shown for activation amplitudes 30%, 36%, and 52%.

of $-3.3 \text{ cal mol}^{-1} \text{ K}^{-1}$ is derived from the ODR procedure, which leads to a ΔS^0_{acid} of 22.7 eu.

Our result is virtually identical to the previous determination of O'Hair et al. obtained by the standard Cooks kinetic method in which entropy effects are omitted.¹ In both cases, however, intramolecular hydrogen bonding and thiolate formation could lead to systematic errors, since these complications are not present in the reference compounds. Moreover, the kinetic method for determining acidities, while widely applied and very precise, is a kinetic approach for the measurement of a thermodynamic quantity. Its accuracy needs to be verified in selected instances, and so, an equilibrium determination also was carried out. This was accomplished by measuring the forward ($k_1 = 2.08 \times 10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$) and reverse ($k_{-1} = 2.76 \times 10^{-9} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$) rates for the proton-transfer reactions shown in eq 1 since the equilibrium



constant is given by the ratio of the rate constants ($K = k_1/k_{-1}$). In the latter case, a correction factor was needed for the pressure measurement of cysteine to obtain k_{-1} because it is not very volatile and was added $\sim 1 \text{ cm}$ from the reaction region but $\sim 1 \text{ meter}$ from where its concentration was determined. This was accomplished by also measuring the proton-transfer rates of cysteine with hydroxide and fluoride ions since these very exothermic processes ($\Delta H^0 = -58$ and $-38 \text{ kcal mol}^{-1}$) safely can be assumed to occur upon every collision.¹⁰ The resulting rate is essentially the same as the collision limit ($k_{ADO} = 2.49 \times 10^{-9} \text{ cm}^3 \text{ molecule}^{-1}$), which is not surprising since this is the exothermic direction. An equilibrium constant of 0.0752 results and leads to $\Delta\Delta G^0_{acid} = 1.5 \pm 0.6 \text{ kcal mol}^{-1}$, where an error of $\pm 100\%$ was adopted for K in the data analysis. This can be combined with $\Delta G^0_{acid}(\text{ClCH}_2\text{CO}_2\text{H}) = 329.0 \pm$

(35) An error of $\pm 2 \text{ eu}$ was assumed for ΔS^0_{acid} , and a similar value of 23.8 is obtained using B3LYP/6-31G(d) geometries and unscaled vibrational frequencies. If the k_{ADO} rate is used instead of k_{-1} in determining the acidity, the reported value is unaffected.

$2.0 \text{ kcal mol}^{-1}$ and a calculated $\Delta S^0_{acid}(\text{cysteine}) = 23.0 \text{ cal mol}^{-1} \text{ K}^{-1}$ using B3LYP/aug-cc-pvdz geometries and vibrational frequencies to give $\Delta G^0_{acid}(\text{cysteine}) = 327.5 \pm 2.1 \text{ kcal mol}^{-1}$ and $\Delta H^0_{acid}(\text{cysteine}) = 334.4 \pm 2.2 \text{ kcal mol}^{-1}$.³⁵ This value is $1.5 \text{ kcal mol}^{-1}$ larger than the kinetic method determination and could reflect a small systematic error in the latter methodology, but the two values are within the experimental uncertainty of either measurement and are well reproduced by B3LYP/6-311++G(d,p)/B3LYP/6-31+G(d), B3LYP/aug-cc-pvdz, and G3B3 predictions of 331.4, 332.3, and 333.3 kcal mol^{-1} , respectively.³² The extended kinetic method is thus able to give reliable thermochemistry even in this case where the reference compounds are carbon and oxygen acids and the substrate of interest is a sulfur acid.

Conclusions

The conjugate base of cysteine undergoes three H/D exchanges with a variety of deuterated alcohols. A conventional mechanism involving sequential proton/deuteron-transfer reactions can be ruled out since ΔH^0_{acid} between cysteine and the deuterated reagents is as large as $43.9 \pm 2.4 \text{ kcal mol}^{-1}$. A relay mechanism involving a zwitterionic intermediate is proposed instead, as it accounts for the observed three H/D exchanges and explains why the carboxyl and amino groups react at the same rate. This pathway requires that the acidities of the thiol and carboxyl positions in cysteine are similar, which is confirmed by DFT and high-level ab initio computations. These calculations also reproduce our measurements of the acidity via the extended kinetic method ($332.9 \pm 3.3 \text{ kcal mol}^{-1}$) and an equilibrium determination ($334.4 \pm 2.2 \text{ kcal mol}^{-1}$) in that B3LYP/aug-cc-pvdz and G3B3 predictions are 332.3 and 333.3 kcal mol^{-1} , respectively. Moreover, they clearly indicate that the thermodynamic deprotonation of cysteine in the gas phase affords a thiolate and not a carboxylate ion. This is a surprising result and indicates that the answer to the title question is no, not in all cases. Cysteine could be unique in this regard, but there is no reason to suppose that this is the case, and preliminary studies indicate that the conjugate base of tyrosine is not just a carboxylate ion.

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Supporting Information Available: Computed B3LYP/aug-cc-pvdz geometries in the form of xyz coordinates and energies for cysteine, its conjugate base, and structures (intermediates and transition states) involved in the H/D exchange process with ethanol, two reaction coordinates for the exchange process, experimental rate constants and graphical examples of the data fits, and complete ref 22. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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