Observation of Cysteine Thiolate and −S···H−O Intermolecular Hydrogen Bond

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The cysteine anion was produced in the gas phase by electrospray ionization and investigated by photoelectron spectroscopy at low temperature (70 K). The cysteine anion was found to exhibit the thiolate form [$^-$SCH$_2$CH(NH$_2$)CO$_2$H], rather than the expected carboxylate form [HSCH$_2$CH(NH$_2$)CO$_2$$^-$]. This observation was confirmed by two control experiments, that is, methyl cysteine [CH$_3$SCH$_2$CH(NH$_2$)CO$_2$$^-$] and cysteine methyl ester [$^-$SCH$_2$CH(NH$_2$)CO$_2$CH$_3$]. The electron binding energy of [$^-$SCH$_2$CH(NH$_2$)CO$_2$H] was measured to be about 0.7 eV blue-shifted relative to [$^-$SCH$_2$CH(NH$_2$)CO$_2$CH$_3$] due to the formation of an intramolecular $-$S$^-$···HO$_2$C$-$ hydrogen bond in the cysteine thiolate. Theoretical calculations at the CCSD(T)/6-311++G-(2df,p) and B3LYP/6-311++G(2df,p) levels were carried out to estimate the strength of this intramolecular $-$S$^-$···HO$_2$C$-$ hydrogen bond. Combining experimental measurements and theoretical calculations yielded an estimated value of 16.4 ± 2.0 kcal/mol for the $-$S$^-$···HO$_2$C$-$ intramolecular hydrogen-bond strength.

1. Introduction

Charged groups are known to play an important role in protein biochemistry.[1−3] In particular, the cysteinate is a protein active site, and because of its highly nucleophilic thiol group, one of the primary functions of cysteine is to act as a nucleophilic catalyst. It is well-known that cysteine is the key catalytic residue within the cysteine proteases such as caspases or papains. Cysteine is also invloved in the activity of many enzymes, protein−protein interactions, or protein−nucleic acid reactions.[4−6] For example, in the conversion of nucleotides to deoxynucleotides, it has been postulated that multiple cysteines play a key role in the reduction process.[7] During the catalysis process, cysteine is in the thiolate form ($^-$SCH$_2$$-$) in order to act as a nucleophile; that is, the sulfur side chain is deprotonated.

While the cysteine anion is known to be important, one of its intrinsic features has not been very well studied: the intramolecular hydrogen bond. The study of hydrogen bonding has long been a focus of extensive research in the fields of chemistry and biochemistry.[8] It is well-known that hydrogen bonding is one of the most important interactions that contribute to the structures and stability of many biological systems. In protein tyrosine phosphatase, the hydrolysis of proteins phosphorylated at tyrosine residues is enhanced by hydrogen bonding in transition-state stabilization.[9] Interestingly, this process also begins with a nucleophilic attack by a cysteine thiolate anion. Recently, there has also been some controversy over the presence and possible importance of short strong low-barrier hydrogen bonds in the transition state of enzymatic catalysis.[10] It has been suggested that the hydrogen bond can provide up to 20 kcal/mol stabilization energy.

Gas-phase photoelectron spectroscopy is a powerful technique to probe the electronic structure and chemical bonding of solution-phase molecules without the perturbation of solvent, yielding intrinsic properties of the solutes studied. Using a newly developed low-temperature electrospray ionization photoelectron spectroscopy (ESI-PES) apparatus, we have recently estimated the low-barrier hydrogen-bond strength in hydrogen maleate.[11] We also investigated previously the effects of hydrogen bonding on the redox potential of [4Fe-4S] centers,[12] which are the active sites of many [Fe-S] proteins.

In this study, we report an investigation of the cysteine anion in the gas phase. A gas-phase acidity measurement of cysteine has been reported previously, where it was suggested that the cysteine anion is deprotonated at the carboxylic acid side.[13] From the pK_a values of the carboxylic acid group (∼2.0) and the side chain thiol group (∼8.2) in cysteine, the anionic form of cysteine should be the carboxylate but not thiolate in the solution phase. However, we found surprisingly that the cysteine anion prefers to be in the thiolate form in the gas phase. Furthermore, an intramolecular hydrogen bond was found to exist between the carboxylic acid $-$CO$_2$H and the thiolate $-$S$^-$.

2. Experimental and Theoretical Methods

2.1. Low-Temperature Photoelectron Spectroscopy. The low-temperature ESI-PES apparatus has been described in several recent studies.[11,14] A key feature of the new ESI-PES apparatus is a temperature-controlled ion trap that is used for ion accumulation and cooling. The ion trap is attached to the cold head of a closed-cycle helium refrigerator, which can reach down to a low temperature of 10 K and can be controlled up to 350 K. The anions of interest, [$^-$SCH$_2$CH(NH$_2$)CO$_2$CH$_3$], [$^-$SCH$_2$CH(NH$_2$)CO$_2$H], and [CH$_3$SCH$_2$CH(NH$_2$)CO$_2$$^-$], were produced by electrospray of 1 mM solutions of the corresponding acid in a mixture of methanol/water solvent (3/1 volume ratio). Anions produced were guided by a RF-only octopole into a quadruple mass filter operated in the RF-only mode. Following the mass filter, ions were directed by a 90° ion bender to the temperature-controlled ion trap, where they were accumulated

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and cooled via collisions with a background gas. The background gas used was ~0.1 mTorr of N_2 for the temperature range between 70 and 350 K. Ions were trapped and cooled for a period of 20−80 ms before being pulsed into the extraction zone of a time-of-flight mass spectrometer at a repetition rate of 10 Hz.

During the PES experiment, ions were mass-selected and decelerated before being intercepted by a probe laser beam in the photodetachment zone of the magnetic-bottle photoelectron analyzer. In the current experiment, photodetachment energies of 4.661 eV (266 nm) and 3.496 eV (355 nm) from an Nd: YAG laser were used. The laser was operated at a 20 Hz repetition rate with the ion beam off at alternating laser shots for shot-by-shot background subtraction. Photoelectrons were collected at nearly 100% efficiency by the magnetic bottle and analyzed in a 5.2-m-long electron flight tube. Time-of-flight photoelectron spectra were collected and converted to kinetic energy spectra, calibrated by the known spectra of I^- and ClO_2^-.[15] The electron binding energy spectra were obtained by subtracting the kinetic energy spectra from the detachment photon energy used. The energy resolution ($\Delta E/E$) was about 2%, that is, ~20 meV for 1 eV electrons.

2.2. Theoretical Methods. Density functional theory (DFT) calculations were used to determine the geometry and electronic structure of the cysteine thiolate [$^-SCH_2CH(NH_2)CO_2H$], cysteine ester [$^-SCH_2CH(NH_2)CO_2CH_3$], and their corresponding neutrals. Geometries were optimized at the B3LYP/6-311++G-(2df,p) level using the Gaussian 03 package of programs,[16,17] and subsequent single-point energy calculations were done at the CCSD(T)/6-311++G(2df,p) level with Molpro2002.6.[18] The CCSD(T) electronic energies were corrected with zero-point vibrational energy (ZPVE) using the vibrational frequencies calculated at the B3LYP/6-311++G(2df,p) level. The adiabatic detachment energies (ADEs) of the anions were obtained by taking the energy difference between the anions and the ground state of the neutrals in their optimized geometries. To estimate the hydrogen-bond strength, we also performed the same procedures on the non-hydrogen-bonded [$^-SCH_2CH(NH_2)CO_2H$] reference structure.

3. Results and Discussion

3.1. Photoelectron Spectra. Figure 1 shows the 266 nm (4.661 eV) photoelectron spectra of the cysteine anion, compared to methyl cysteine [$CH_3SCH_2CH(NH_2)CO_2^-$] and cysteine methyl ester [$^-SCH_2CH(NH_2)CO_2CH_3$]. Because no photodetachment transition occurs below 2.0 eV, all the spectra are plotted from 2.0 to 4.6 eV binding energy scale for clearer presentation. There are two possible sites of cysteine deprotonation, either from the thiol or from the carboxylic acid group. From our previous experiences on the PES of different carboxylate anions,[14] the binding energy of the CO_2^- group (without external interactions) is about ~3.4 eV. There is little dependence on the side chains attached to the CO_2^- group because photodetachment primarily occurs from the $-CO_2^-$ derived molecular orbitals.[11,14,19] The photoelectron spectrum of [$CH_3SCH_2CH(NH_2)CO_2^-$] in Figure 1a indeed shows an electron binding energy onset at around 3.4 eV, consistent with detachment from a $-CO_2^-$ group. If the cysteine anion is in the carboxylate form [$HSCH_2CH(NH_2)CO_2^-$], the binding energy is expected to be ≥3.4 eV. However, as shown in Figure 1b, the binding energy of the cysteine anion is about 3.1 eV only, suggesting that the cysteine anion does not contain a $-CO_2^-$ group and is more likely in the thiolate form [$^-SCH_2CH(NH_2)CO_2H$].
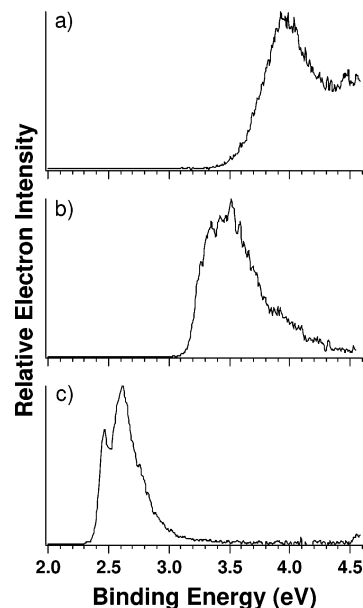

Figure 1. Photoelectron spectra of (a) methyl cysteine [$CH_3SCH_2CH(NH_2)CO_2^-$], (b) cysteine thiolate [$^-SCH_2CH(NH_2)CO_2H$], and (c) cysteine methyl ester [$^-SCH_2CH(NH_2)CO_2CH_3$], measured at 266 nm (4.661 eV) and 70 K ion trap temperature.

To investigate this further, we performed another control experiment using the cysteine methyl ester [$^-SCH_2CH(NH_2)$-CO_2CH_3], as shown in Figure 1c. The 2.4 eV binding energy is close to the literature values for a series of thiolate anions [$^-S(CH_2)_nCH_3$] ($n = 0-4$), ranging from ~1.8 ($n = 0$) to ~2.1 ($n = 4$) eV.[20] This suggested that the chain length has only minor effects on the binding energy of RS^- because the charge is localized at the sulfur atom, albeit there is a trend that longer chain length induces higher binding energies. However, the binding energy of cysteine thiolate [$^-SCH_2CH(NH_2)CO_2H$] [Figure 1b] is significantly blue-shifted compared to that of [$^-SCH_2CH(NH_2)CO_2CH_3$]. The blue shift suggested the formation of intramolecular hydrogen bonding between the $-CO_2H$ group and $-S^-$, which would greatly stabilize the anion. Partially resolved vibrational structures were observed in the spectra of both [$^-SCH_2CH(NH_2)CO_2H$] and [$^-SCH_2CH(NH_2)$-CO_2CH_3] whereas the [$CH_3SCH_2CH(NH_2)CO_2^-$] spectrum was broad and featureless.

Higher resolution 355 nm (3.496 eV) photoelectron spectra of cysteine thiolate [$^-SCH_2CH(NH_2)CO_2H$] and cysteine methyl ester [$^-SCH_2CH(NH_2)CO_2CH_3$] are shown in Figure 2. Because of the elimination of vibrational hot bands in the current low-temperature conditions, the long tail at the low binding energy side suggested that some low-frequency vibrational modes might be active during photodetachment. In particular, because both [$^-SCH_2CH(NH_2)CO_2CH_3$] and [$^-SCH_2CH(NH_2)CO_2H$] possess no symmetry, many low-frequency vibrational modes could possibly become excited during the photodetachment process. From our theoretical calculations, there are 12 and 9 vibrational modes in [$^-SCH_2CH(NH_2)CO_2CH_3$] and [$^-SCH_2CH(NH_2)CO_2H$] with frequencies lower than 600 cm^{-1}, respectively. Due to the low signal-to-noise ratio and lack of vibrational resolution in the lower binding energy side, we were only able to estimate the threshold detachment energy (TDE) from the onset of appreciable photoelectron signals. The TDE of [$^-SCH_2CH(NH_2)$-CO_2CH_3] and [$^-SCH_2CH(NH_2)CO_2H$] are estimated to be 2.36 and 3.07 eV, respectively. The difference in TDE values of [$^-SCH_2CH(NH_2)CO_2CH_3$] and [$^-SCH_2CH(NH_2)CO_2H$] is 0.71 eV (16.4 kcal/mol), which can be viewed as the intramolecular
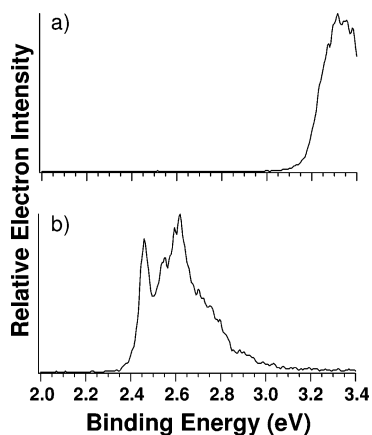
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Figure 2. Photoelectron spectra of (a) cysteine thiolate [$^-$SCH$_2$CH(NH$_2$)-CO$_2$H] and (b) cysteine methyl ester [$^-$SCH$_2$CH(NH$_2$)CO$_2$CH$_3$], measured at 355 nm (3.496 eV) and 70 K ion trap temperature.

hydrogen-bond strength in the cysteine thiolate. This estimate is based on three assumptions: (1) the photodetachment process in both [$^-$SCH$_2$CH(NH$_2$)CO$_2$CH$_3$] and [$^-$SCH$_2$CH(NH$_2$)CO$_2$H] is similar; (2) the intramolecular hydrogen bonds in [$^-$SCH$_2$CH-(NH$_2$)CO$_2$CH$_3$] and neutral [SCH$_2$CH(NH$_2$)CO$_2$CH$_3$] are negligible; and (3) the hydrogen-bond strength in [SCH$_2$CH(NH$_2$)-CO$_2$H] neutral is negligible. The first assumption should be valid on the basis of the fact that the charges are localized at the thiolate S$^-$ in both anions. The second assumption is supported by the fact that the 70 K spectrum of [$^-$SCH$_2$CH(NH$_2$)CO$_2$CH$_3$] did not have an observable binding energy shift when compared to its room-temperature spectra (not shown),[14a] which also means that the intramolecular hydrogen bonding in the [SCH$_2$CH-(NH$_2$)CO$_2$CH$_3$] neutral is negligible. The third assumption can be verified by the geometrical parameters of the [SCH$_2$CH-(NH$_2$)CO$_2$H] neutral (see below).

3.2. Theoretical Calculations. To gain further insight into the stability of the cysteine thiolate in the gas phase and its intramolecular hydrogen bonding, theoretical calculations were performed. The single-point energy of cysteine carboxylate is calculated to be about 0.10 eV higher in energy than cysteine thiolate at the CCSD(T)/6-311++G(2df,p) level (Table 1). Although the energy difference is relatively small, the calculation indeed confirmed that cysteine thiolate is more stable than cysteine carboxylate. In a recent reactivity study of phenyl radicals with cysteine and methionine, it was found that the product branching ratio of hydrogen abstraction from cysteine is much higher than methionine.[21] It was suggested that the majority of the hydrogen abstraction comes from the SH group in cysteine; that is, when gaseous cysteine neutral reacts with phenyl radical, the sulfur hydrogen is the preferred site for hydrogen abstraction. This reactivity measurement provides alternative experimental evidence for the formation of cysteine thiolate in the gas phase.

Figure 3. Optimized geometries of (a) [$^-$SCH$_2$CH(NH$_2$)CO$_2$CH$_3$] and [SCH$_2$CH(NH$_2$)CO$_2$CH$_3$], (b) [$^-$SCH$_2$CH(NH$_2$)CO$_2$H] and [SCH$_2$CH-(NH$_2$)CO$_2$H], and (c) non-hydrogen-bonded reference structure [$^-$SCH$_2$CH(NH$_2$)CO$_2$H]. Selected bond lengths are given in angstroms; −CHS and −OHS angles are given in degrees. Yellow, S; gray, C; red, O; blue, N; cyan, H.

The calculated ADEs of [$^-$SCH$_2$CH(NH$_2$)CO$_2$CH$_3$] and [$^-$SCH$_2$CH(NH$_2$)CO$_2$H] at CCSD(T)/6-311++G(2df,p) level are 2.31 and 2.96 eV, respectively (Table 1). Both theoretical ADEs are lower than the experimental TDEs. The theoretical ADE difference gives the value of 0.65 eV (15.0 kcal/mol) on the hydrogen-bond strength, in good agreement with the experimental observation. Theoretically, the hydrogen-bond strength can also be estimated by the choice of a reference structure (non-hydrogen-bonded [$^-$SCH$_2$CH(NH$_2$)CO$_2$H] isomer).[22] The reference structure was optimized with the carboxylic acid flipped away from the thiolate group so that no

TABLE 1: Experimental and Theoretical Adiabatic Detachment Energies (ADEs) of $^-$SCH$_2$CH(NH$_2$)CO$_2$H and $^-$SCH$_2$CH(NH$_2$)CO$_2$CH$_3$, as Well as the Energies of HSCH$_2$CH(NH$_2$)CO$_2^-$ and the Non-Hydrogen-Bonded $^-$SCH$_2$CH(NH$_2$)CO$_2$H Relative to the Hydrogen-Bonded $^-$SCH$_2$CH(NH$_2$)CO$_2$H

	ADE (eV)		energy relative to $^-$SCH$_2$CH(NH$_2$)CO$_2$H (eV)
	exp[a]	theo	
$^-$SCH$_2$CH(NH$_2$)CO$_2$H	3.07(5)[b]	3.06[c], 2.96[d]	0.00
HSCH$_2$CH(NH$_2$)CO$_2^-$			0.07[c], 0.10[d]
$^-$SCH$_2$CH(NH$_2$)CO$_2$H (non-hydrogen-bonded)			0.79[c], 0.76[d]
$^-$SCH$_2$CH(NH$_2$)CO$_2$CH$_3$	2.36(5)[b]	2.41[c], 2.31[d]	

[a] Numbers in parentheses are uncertainties in the last digits. [b] Threshold detachment energy (TDE), representing the upper limit for the ADE. [c] Computed at B3LYP/6-311++G(2df,p)//B3LYP/6-311++G(2df,p) level plus ZPVE corrections at B3LYP/6-311++G(2df,p) level. [d] Computed at CCSD(T)/6-311++G(2df,p)//B3LYP/6-311++G(2df,p) level plus ZPVE corrections at B3LYP/6-311++G(2df,p) level.
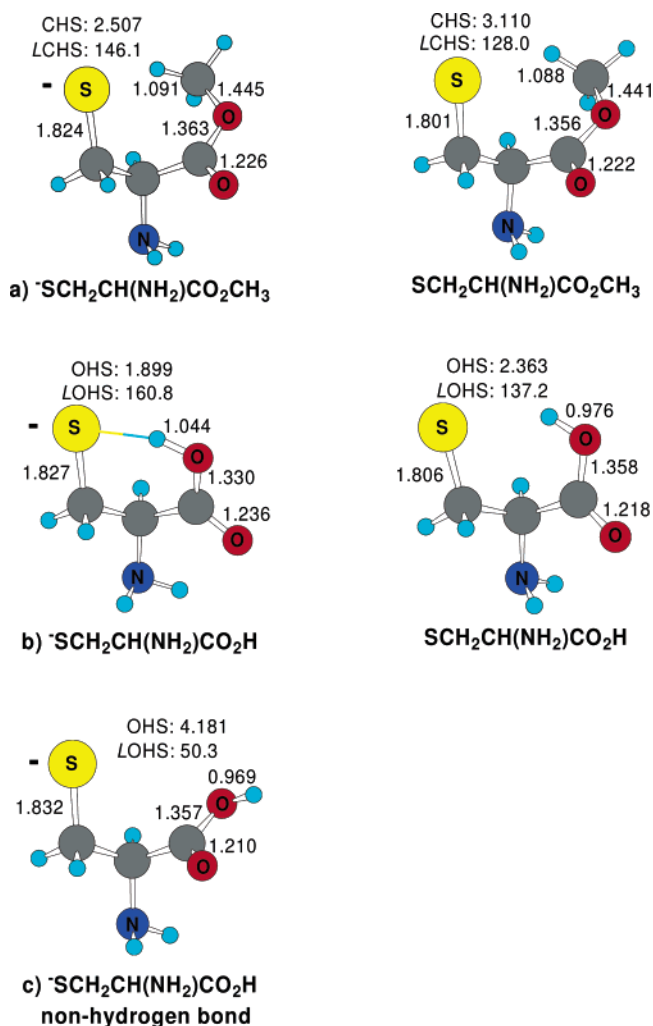
hydrogen bond can be formed between the $-CO_2H$ and $-S^-$ groups. This non-hydrogen-bonded reference structure exhibits only minimal structural difference from the hydrogen-bonded $[^-SCH_2CH(NH_2)CO_2H]$, except that the hydrogen is flipped and the carboxylic group is rotated.

The optimized geometries of $[^-SCH_2CH(NH_2)CO_2CH_3]$ and $[^-SCH_2CH(NH_2)CO_2H]$, as well as their corresponding neutrals and the non-hydrogen-bonded $[^-SCH_2CH(NH_2)CO_2H]$, are shown in Figure 3. We observed a rather big geometrical change in the $O-H\cdots S$ distance and $-OHS$ angle between the cysteine thiolate $[^-SCH_2CH(NH_2)CO_2H]$ and its neutral $[SCH_2CH(NH_2)CO_2H]$. The $O-H\cdots S$ distance is significantly increased from 1.899 to 2.363 Å and the $-OHS$ angle is decreased from 160.8° to 137.2°. From the proposed geometrical criteria of a hydrogen bond,[23a] the hydrogen-bond strength in $[SCH_2CH(NH_2)CO_2H]$ is considered to be weak. In particular, the $-OHS$ angle was rarely observed to be $< 145°$ in other $O-H\cdots S$ hydrogen-bonding systems.[23b] Thus, it can be concluded that the hydrogen-bond strength in the $[SCH_2CH(NH_2)CO_2H]$ neutral is negligible; that is, assumption 3 outlined above is fulfilled. The theoretical estimate at the CCSD(T)/6-311++G(2df,p) level gives a value of 17.5 kcal/mol (0.76 eV, Table 1) for the hydrogen-bond strength of cysteine thiolate, which is taken from the energy difference between $[^-SCH_2CH(NH_2)CO_2H]$ and the non-hydrogen-bonded reference structure.

Experimentally, the TDE difference between $[^-SCH_2CH(NH_2)CO_2CH_3]$ and $[^-SCH_2CH(NH_2)CO_2H]$ should represent a good estimate of the hydrogen-bond strength in cysteine thiolate, given the fact that the hydrogen-bond strength in $[^-SCH_2CH(NH_2)CO_2CH_3]/[SCH_2CH(NH_2)CO_2CH_3]$ and $[SCH_2CH(NH_2)CO_2H]$ neutral is negligible. Interestingly, the experimental estimate (16.4 kcal/mol) falls between the theoretical values for the $[^-SCH_2CH(NH_2)CO_2CH_3]$ reference (15.0 kcal/mol) and the non-hydrogen-bonded reference $[^-SCH_2CH(NH_2)CO_2H]$ (17.5 kcal/mol). Considering the uncertainties in the experiments and theoretical calculations, our best estimate for the hydrogen-bond strength in cysteine thiolate is 16.4 ± 2.0 kcal/mol, which is comparable to other $O-H\cdots S^-$ hydrogen-bond strengths.[24]

4. Conclusions

In summary, the photoelectron spectrum of cysteine anion was measured at 70 K. The cysteine anion was found to exists as a hydrogen-bonded thiolate $[^-SCH_2CH(NH_2)CO_2H]$ rather than a carboxylate $[HSCH_2CH(NH_2)CO_2^-]$. The intramolecular $O-H\cdots S^-$ hydrogen-bond strength in cysteine thiolate anion was estimated experimentally as 16.4 ± 2.0 kcal/mol.

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