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Re-print of "Gas-phase acid–base properties of homocysteine, homoserine, 5-mercaptonorvaline and 5-hydroxynorvaline from the extended kinetic method"☆

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ABSTRACT

The gas-phase proton affinities (PA) and gas-phase acidities (GA) for the non-protein amino acids homocysteine (hcys), homoserine (hser), 5-mercaptonorvaline (hhcys), and 5-hydroxynorvaline (hhser) have been determined using the extended kinetic method in an ESI-triple quadrupole instrument. Increasing the length of the side chain of cysteine by one and two methylene units has a dramatic effect on the proton affinity. The PAs of hcys and hhcys were determined to be 932 ± 9 kJ/mol and 944 ± 9 kJ/mol, 29 and 41 kJ/mol more basic than cysteine. The proton affinity enhancement for the serine homologs is more dramatic. PAs of 951 ± 11 and 986 ± 10 kJ/mol were measured for hser and hhser, 39 and 74 kJ/mol more basic than serine. The proton affinity enhancement can be rationalized by the increased stability of internally hydrogen-bonded structures in the cations of the homo-amino acids with longer side chains. High-level density functional theory calculations confirm the cyclic structures for the cations and give predictions for the proton affinities that are in excellent agreement with the measured results. The GAs for hcys was determined to be 1398 ± 10 kJ/mol which is the same as the GA of cysteine (1396 ± 9 kJ/mol). Calculations predict that as with cysteine, the preferred site of deprotonation for hcys is the sulfur atom in the side chain rather than the carboxylic acid group. For hhcys, the lowest enthalpy structure is an internally-hydrogen-bonded thiolate, while the lowest free energy structure is a carboxylate with an extended side chain. These conformers lie within 5 kJ/mol of each other in both enthalpy and free energy and should both be populated in a gas-phase sample. Theoretical predictions for the acidities of hcys and hhcys predict a slight trend in acidity with increasing side-chain length. The experimental GA for hser was determined to be 1394 ± 10, the same as serine (1393 kJ/mol), and calculations predict that Ser, hser, and hhser all have the same acidity within error.

1. Introduction

In the hundred years since J.J. Thompson's discovery of the electron, mass spectrometry has become one of the most important chemical analysis tools [1]. In addition to its uses in isotope enrichment and separation [2], identification of organic compounds [3,4] and proteomics applications [5–9], mass spectrometry is second to none as a technique for determining reliable thermochemical quantities for ionic and neutral species [10–19]. The versatility of modern ionization sources has allowed for the determination of thermochemical properties of organic, organometallic, inorganic, and, more recently, biological compounds. The advent of FAB [20], electrospray [21], and MALDI [22] soft ionization sources has allowed for the study of non-volatile biological species, such as peptides and proteins using both low-resolution/unit mass and high-resolution/exact mass spectrometers.

Among the first thermochemical measurements made on non-volatile biological species were the determination of the absolute proton affinities (PA) and gas phase acidities (ΔH_{acid}, GA) of the 20 common "protein amino acids" (PAA). Pioneering work by Locke and McIver [23,24] Kebarle [25], Amster [26], Bojesen [27,28],

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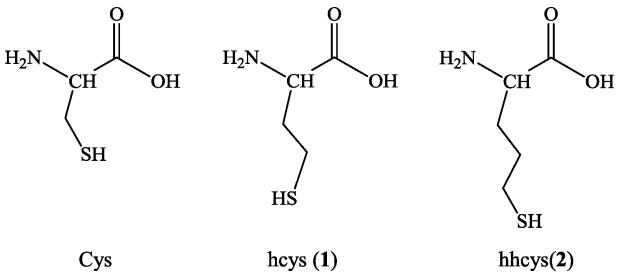
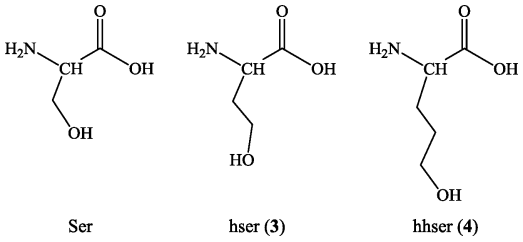
Harrison [29], Tabet [30], O'Hair, Gronert, and Bowie [31], Cassady [32–35], and Bouchoux [36–45], using a variety of experimental and theoretical methods, established thermochemical values for most of the PAAs. One difficulty with measuring acid-base properties of non-volatile species is that it is quite difficult to generate sufficient, quantifiable pressures of the neutral species for use in gas-phase equilibrium or two-way bracketing studies [46]. As a result, much of the recent work on the acid-base properties of amino acids makes use of the Cooks' kinetic method, which relies on competitive cleavage of proton-bound dimer ions to give thermochemical quantities [10,47]. These proton-bound dimer ions can be formed directly from FAB or ESI sources, without the need for generating pressures of neutral non-volatile species. The proton-bound dimer ions of analyte of interest and one of a series of reference compound are dissociated in a tandem mass spectrometer and the ratio of the resulting product ions is used to extract thermochemical properties. In an extended kinetic method analysis, the dissociations are repeated at multiple collision energies, which allowed the de-convolution of enthalpic and entropic quantities [48–51].

We have been using the extended kinetic method to measure proton affinities and gas-phase acidities for a class of compounds known as "non-protein amino acids" (NPAA) [52–56]. NPAAs are not coded for by RNA, but are found throughout nature as secondary products of plant and fungi metabolism [57]. Many NPAAs are similar in structure to one or more of the PAAs and can compete with them in a variety of biological processes, including being misincorporated into proteins [58–64]. In addition to their biological relevance, NPAAs serve as useful model compounds for studying the interplay between amino acid structure and thermochemical properties. We are especially interested in homologous series of amino acids in which the side-chain length is varied systematically. Many of these species can form strong intramolecular hydrogen bonds involving the side chain that depend on ring size and can have varying effects on gas-phase amino acid properties. For example, the proton affinity of lysine (Lys, side chain = $CH_2CH_2CH_2CH_2NH_2$) and its homologs: ornithine (Orn, side chain = $CH_2CH_2CH_2NH_2$), 2,4-diaminobutanoic acid (Daba, side chain = $CH_2CH_2NH_2$), and 2,3-diaminopropanoic acid (Dapa, side chain = CH_2NH_2) were shown to vary systematically with side-chain length [54]. In addition, the hydrogen–deuterium exchange rates with D_2O for these species were also shown to vary with ring size, with protonated orn exchanging nearly 200 times slower than the other homologs [65]. Density functional theory calculations showed that ornithine forms a strong seven-membered intramolecular hydrogen bond between the side chain and the amino group. This H-bond is especially strong and resists the insertion of a D_2O molecule, as required for HDX through the relay mechanism. In contrast to the positive ion results, we recently showed that the gas phase acidity (ΔH_{acid}) for the lysine homologs was the same for all four homologs, despite the anionic structures containing intramolecular hydrogen bonds between the side-chain and the COO_2^- group in Daba and Dapa [55].

In this manuscript, we extend our studies of NPAAs with varying side-chain lengths to those containing SH and OH groups. Cysteine (Scheme 1) is an important PAA as it can form di-sulfide bonds with other Cys residues, that contribute to the stability and structure of proteins. The PA for cysteine was determined by Harrison to be 904 kJ/mol using the kinetic method [66]. A recent review by Bouchoux and co-workers recommends as PA for Cys of 903 kJ/mol based on an evaluation of experimental and a variety of high-level theoretical calculations [42]. The gas-phase acidity for Cys was determined by Poutsma and Kass to be 1393 ± 14 kJ/mol using both the kinetic method and gas-phase equilibrium techniques [67]. The solution-phase pK_a values for the COOH and SH groups are *ca.* > 3.1 and 8.5, respectively, indicating that deprotonation in solution is preferred at the COOH site [68]. As initially noted by Wang and co-workers in their photo-electron spectroscopy study of deprotonated cysteine, gas-phase cysteine has a thiolate structure [69]. The thiolate nature of deprotonated cysteine has now been established by several groups using both experimental and theoretical methods [42,69–72]. The IRMPD data of Oomens et al. in which they see no evidence for the thiolate ion [73] has since been rationalized by the fact that their ion source generates predominantly solution-like structures, which would be carboxylate conformers [74]. Bouchoux recommends a ΔH_{acid} of 1395 for Cys [42]. Serine (scheme 2) is also an important amino acid as its OH side chain group can be phosphorylated as part of cell signaling pathways in cells [68]. The PA of Ser was determined to be 910 kJ/mol by Harrison and Bouchoux recommends an evaluated PA of 912 kJ/mol. ΔH_{acid} for Ser was determined to be 1391 kJ/mol by our group using the extended kinetic method in an ion trap mass spectrometer [75]. We present here a systematic study of the proton affinities and gas-phase acidities for the homocysteine (hcys, **1**), 5-mercaptonorvaline (hhcys, **2**), homoserine (hser, **3**), and 5-hydroxynorvaline (hhser, **4**) using the extended kinetic method in a triple quadrupole mass spectrometer. Isodesmic hybrid density functional theory calculations at the B3LYP/6-311++G(d,p)//B3LYP/6-31+G(d) level of theory are also presented that support the experimental studies and provide relative acidities of the SH and COOH groups in hcys and hhcys.



Scheme 1. Chemical structures of cysteine (cys), homocysteine (1, hcys), and 5-mercaptonorvaline (hhcys, 2).



Scheme 2. Chemical structures of serine (Ser), homoserine (3, hser), and 5-hydroxynorvaline (hhser, 4).

2. Experimental

All experiments were performed in a Thermo TSQ Quantum Discovery triple quadrupole instrument. Full experimental details have been presented elsewhere [55]. Briefly, dilute solutions (*ca.* $1–5 \times 10^{-4}$ M) an amino acid and one of a series of reference bases in slightly acidified (1% HOAc) 50:50 methanol:water are directly infused (flow rates 5–15 μl/min) into the electrospray ionization source of the TSQ. Electrospray and ion focusing conditions were also varied to maximize the ion count for the proton-bound heterodimer $[A–H^+–B_i]^+$. These ions are isolated in Q1 at a resolution of 0.7 amu and are allowed to pass into the rf-only collision cell (q2). The isolated ions are allowed to undergo collision-induced dissociation with argon gas maintained at a pressure of 0.3 mTorr. Product ion spectra are recorded at collision energies between 0 and 30 V (lab). The ion intensities of each primary product and any secondary