Reactions between neutral molecules and cation-radicals in the gas-phase: Can protonation occur without proton transfer?

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ABSTRACT

Gas-phase collisions of Gly and Ala with free electrons were employed to form positive ions at well determined energies. At elevated molecular vapor pressure, protonated $[Gly+H]^+$ and $[Ala+H]^+$ ions were also observed. The appearance energies of $[Gly+H]^+$ and $[Ala+H]^+$ were both determined as 0.30 ± 0.14 eV higher than the ionization energies of their $M^{\bullet+}$ congeners. Quantum chemical calculations predict proton transfer reactions rather than hydrogen atom abstraction reactions as the likely $[M+H]^+$ forming method in our experiment. Microcanonical rate coefficient calculations using B3LYP/6-311++G(2d,p) frequencies and rotational constants, support abstraction of a C_{alpha} proton for glycine, whereas the C-terminal proton abstraction mechanism was found as being more favorable for alanine. Additionally, substantial immonium ion formation was observed at experimental energies identical to either the protonated product or cation radical for glycine and alanine, respectively. Theoretical barriers to $M^{\bullet+}$ cation radical fragmentation to $HRCNH_2^+$ indicate that they are product-limited and that the glycine barriers are generally larger than those for alanine.

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1. Introduction

Cross-sections for gas-phase ion-molecule collisions have long been recognized as being controlled by Langevin-type long-range interaction potential [1]. Deviations from this behavior for exo- and endothermic reactions have recently been considered at length [2]. Proton/hydrogen exchanges that accompany many ion-molecule collisions are typical examples of these interactions and they are well described in terms of Brønsted and Lewis acid–bases formalism [3]. Since the first observation of methonium ion CH_5^+ by Tal'roze and Ljubimova [4], protonation phenomenon has become a subject of intensive study in mass spectrometry (MS), playing a key role in chemical ionization [3], CI, and many other MS techniques that deal with protonated species. Although widely used in MS, many aspects of proton/hydrogen transfer resulting in the generation of charged species remain not clearly understood. The most challenging issue in this case is a self-protonation reaction occurring between a neutral molecule and its positive cation-radical [5]. The unexpected observation of $[M+H]^+$ protonated species in

electron ionization (EI) mass spectra is considered a secondary process [4,6] and thus confounds interpretation of experimental data. The first reports on EI mass spectra of common amino acids [7–9] do not mention registration of protonated species, unlike their ester derivatives [10]. However, despite these early reports, EI spectra in the NIST database, clearly indicate the formation of protonated ions for some amino acids [11] has occurred. Majer et al. [12] studying electron ionization of Gly tried to explain the observation of $[M+1]^+$ ions and other higher masses ions via the complex nature of chemical processes occurring during evaporation at high molecular temperatures (M = neutral analyte molecule). From this work it is, however, unclear if protonated species can still be formed also under EI conditions at lower molecular temperatures and to what extent. Another question that requires explanation is whether self-protonation processes occur via hydrogen or proton abstraction. Several theoretical questions regarding the fragmentation of amino acids' cation-radicals [13–16] and intramolecular proton transfer in amino acids [17–20] have been examined in the literature. One should note, however, that while mass spectrometrically observed protonated amino acids might be products of intramolecular reactions, these reactions cannot occur in a single amino acid, but rather proceed within their higher mass charged complexes or as an inter-molecular particle transfer during two-body collisions between charged and neutral amino acids [5]. Recent energy-resolved fragmentation of amino acids' cation-radicals with the determination

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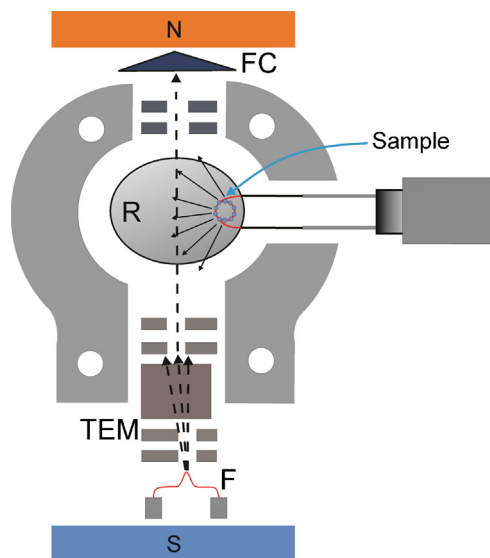
Fig. 1. Schematic of ion chamber with direct exposure probe. F: filament, R: ion repeller, FC: Faraday cup (electron collector), N & S poles of collimating magnet, TEM: trochoidal electron monochromator.

of ionization (IE) and appearance energies (AE) of molecular cation-radicals and fragmented cations, respectively, have been examined experimentally by mass spectrometry [21–23]. In principle, these studies could shed light onto these questions. Nonetheless, there is a discrepancy between these and earlier EI mass spectrometry data [7–9] as well as between them and the data produced by photoelectron spectroscopy [24–28] and photoionization mass spectrometry [29]. In the present work, the EI of the two simplest amino acids, glycine (Gly) and alanine (Ala) was re-examined as part of developmental work on a custom-made trochoidal electron monochromator orthogonal-extraction time-of-flight [30,31] and the behavior was found to differ between the two. This finding prompted us to investigate the practical details of what might be occurring. We have utilized quantum chemical calculations at different levels of theory to aid interpretation of the experimental data.

2. Methods

2.1. Experimental details

A custom-made orthogonal-extraction time-of-flight (oTOF)-MS [30,31] incorporating a trochoidal electron monochromator (TEM) [32] for the generation of an electron beam with an energy spread of 70–200 meV was utilized to produce cation-radicals of Gly and Ala over the 0–75 eV electron energy range. The instrument can handle three-dimensional mass spectra (ion intensity, ion m/z, electron energy) in real time at a very fast rate [31,33] and thereby allows use of a newly-designed custom-made Direct Exposure Probe (DEP). Gly and Ala samples in aqueous and water/methanol solutions were deposited onto the surface of the direct exposure probe's heating spiral (Fig. 1) and dried prior to inserting the probe into the instrument's ionization chamber. For the external calibration, Ar and air were introduced into the ionization chamber to calibrate the mass-to-charge, m/z, and calibrate and control linearity of the electron energy scale. In the experiments, where ionization efficiency curves were recorded, only Ar was present and air was shut off. In some experiments, methanol, when it was not completely vaporized from the DEP, served as an additional calibrant. Electron energy resolution was ca. 100–150 meV. In contrast to previous work on peptides [34] where the probe was heated

by radiation from the surrounding, hot ionization chamber, in the present work a tungsten coil (2 turns) of the DEP was resistively heated to 100–150 °C within less than ~0.5 s to vaporize the intact amino acids generating a high vapor density to secure collisions within the vapor. Temperature of the DEP' wire was calibrated in a separate experiment with a thermocouple connected to the DEP. Close distance of the DEP to the electron beam allowed production of sufficient vapor pressure for recording mass spectra even without heating the DEP (it was not sufficient for recording high quality ionization efficiency curves though). To have a stable ion signal, the ionization efficiency curves for Gly and Ala were recorded at the DEP temperature ca. 100–120 °C. In earlier mass spectrometry publications [7,10], it was also noted that placing solid amino acid directly to the ionization chamber provided adequate sample pressure at lower temperatures than in the case of externally heated inlet systems.

In a similar manner to earlier experiments [34], the position of the DEP relative to the electron beam was critical as ion signal and thus mass spectra were only generated when the probe was proximate to rather than physically in the electron beam. By varying the distance between the DEP's tip and electron beam, and the electron energy it was possible to control ion production to yield either molecular cation-radicals almost exclusively or simultaneously with their protonated congeners. The separation between probe tip and electron beam insured that cation-radicals of the amino acids studied were only formed by EI, as demonstrated by the energy dependencies of the ion production processes observed in both positive and negative ion modes. Mass spectra recorded with the DEP under conditions favoring molecular cation-radicals formation were indistinguishable from those obtained with a conventional direct insertion probe [11] previously. Control experiments performed with the electron beam turned off resulted in no protonated species being detected indicating that protonation likely occurred via processes associated with EI either via collision of the amino acid cation-radicals with their neutral counterparts or via electron ionization of neutral amino acid aggregates. To be certain that positive ions were not formed by pyrolysis, additional experiments were performed with the trochoidal electron monochromator's filament turned off; the result of which was that the ion signals disappeared immediately, thus confirming that sample heating did not produce ions by some pyrolytic action.

2.2. Calculation details

Standard ab initio and density functional theory (DFT) calculations were performed with the Gaussian 09 suite of programs [35]. Electronic and geometrical structures of intact neutral and positively-charged Gly and Ala, and the associated transition structures were examined using the B3LYP [36] and M06-2X [37] models utilizing the 6-311++G(2d,p) basis set. Single point energies were then calculated from the B3LYP/6-311++G(2d,p) minima at the MP2/6-311++G(2d,p) [38] level of theory. The MP2 and B3LYP values were then averaged (B3LYP-MP2) to cancel out known errors in the methods [39]. The spin-unrestricted formalism (UB3LYP, UM06-2X and UMP2) was used for all calculations of radical systems. Stationary points were characterized by harmonic frequency calculations to identify local energy minima (all real frequencies) and first-order saddle points (transition structures, one imaginary frequency). Multiple transition structures (TSs) were investigated for both the hydrogen transfer and proton transfer mechanisms considered here. Intrinsic reaction coordinate (IRC) calculations were run for all barriers to determine which minima the TSs connected and thus define the detailed reaction pathway. The final structures on both the product and reactant sides of the IRC were then optimized with small incremental steps (by utilizing the Gaussian09 keywords IOp(2/9 = 11, 1/8 = 2)).