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Feature article

Revealing the structure of isolated peptides: IR-IR predissociation spectroscopy of protonated triglycine isomers ☆

Jonathan M. Voss, Kaitlyn C. Fischer, Etienne Garand *

Department of Chemistry, University of Wisconsin, 1101 University Avenue, Madison, WI 53706, United States

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ABSTRACT

We report an isomer specific IR-IR double resonance study of the mass-selected protonated triglycine peptide. Comparison of experimental spectra with calculations reveals the presence of two isomers, with protonation occurring at either the terminal amine site or one of the amide oxygen sites. The amine protonated isomer identified in our experiment contains an atypical *cis* amide configuration as well as a more typical *trans* amide. The amide protonated peptide, on the other hand, contains two *trans* amide moieties. Both isomers are found to be the lowest energy structures for their respective protonation site, but it is unclear, from experiments and calculations, which one is the global minimum. The presence of both in our experiments likely points to kinetic trapping of a higher energy structure. Finally, the observed frequencies of the N—H and O—H stretch vibrations are used to estimate the hydrogen-bond strengths present in each isomer, accounting for the relative stabilities of these structures.

1. Introduction

With multiple amide linkages connecting the amine and carboxyl terminals, there are a myriad of intramolecular interactions, such as hydrogen-bonding, present in a peptide. Various combinations of these interactions govern the relative stabilities of different structures, providing the flexibility that allows a peptide to structurally adapt to different environments. Studying these interactions at the molecular level can therefore reveal how three-dimensional structures and functionalities are regulated in larger species in specific environments. For instance, one of the simplest model peptides, protonated triglycine (Gly_3H^+), exhibits some interesting competing stability factors in the gas phase. Observations of protonation at either the amine nitrogen N^1 or amide oxygen O^1 site (see Fig. 1) have been reported [1,2]. The latter is surprising for this small peptide because the gas phase proton affinity of an amide is ~23 kJ/mol less than that of an amine [3], indicating that an amine protonated Gly_3H^+ structure should be considerably more stable. The observation of an O^1-protonated isomer therefore points to the presence of stronger intramolecular interactions that make this structure energetically competitive. Numerous isomers are possible even for this small peptide, and inaccuracies associated with calculating non-covalent interactions make it difficult to predict the exact energetic ordering [4]. Therefore, experimentally probing the precise structure of each isomer can provide valuable insights into their relative stabilities and the strengths of the related intramolecular interactions.

Mass spectrometry (MS) is often used to characterize higher order structures in biological systems, especially in combination with other methods such as ion mobility [5–7], hydrogen/deuterium exchange [8–10], or chemical cross-linking [11,12]. In such experiments, electrospray ionization (ESI) [13,14] is generally employed to gently transfer the species of interest to the gas phase. Moreover, by combining vibrational spectroscopy with MS, it is possible to probe the structures and specific non-covalent interactions present in a peptide. Such studies have been carried out via infrared multiple photon dissociation (IRMPD) spectroscopy [1,15–19], UV-IR double resonance spectroscopy [20–23], and cryogenic ion vibrational predissociation spectroscopy (CIVS) [24–27]. In previous studies [1,2] of the Gly_3H^+ system via IRMPD in the 1050–1900 cm^{-1} region, the results indicated the presence of at least two isomers, one with a bent N^1-protonated structure and another linear structure with protonation occurring between adjacent amide C=O groups.

Here, we present the isomer-specific IR-IR double resonance CIVS spectroscopy of protonated triglycine. The IR-IR approach is an extension of CIVS that allows for isomer selective spectroscopy of any mass-selected ions [24,28]. Moreover, the use of a cryogenic ion trap and weakly-bound D_2 as messenger tags ensure low

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* Corresponding author.
E-mail address: egarand@wisc.edu (E. Garand).

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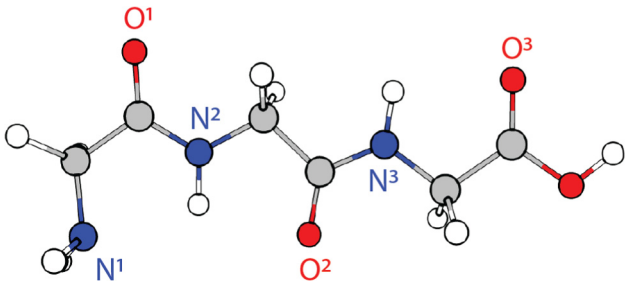

Fig. 1. An example of a neutral triglycine molecule showing the numbering scheme of N and O groups starting from the amine terminal.

internal energies in the Gly_3H^+ ions, minimizing spectral broadening and congestion, as well as reducing the number of isomers present. The resulting isomer-specific vibrational spectra in a broad 1000–3800 cm^{-1} spectral window provide unambiguous identification of the structures present. We further characterize the N—H and O—H (hydrogen-bond donor) stretch vibrations to reveal the strength of the intramolecular interactions present in each isomer.

2. Experimental and calculation details

The infrared spectra of Gly_3H^+ were obtained using our home-built cryogenic ion vibrational spectrometer described in detail previously [29]. Briefly, ESI of a ~1 mM solution of triglycine in methanol with a trace amount of formic acid was used to generate the Gly_3H^+ ions. The ions were transferred through several differentially pumped regions by a series of hexapole ion guides and biased apertures into a 3D quadrupole ion trap held at 10 K by a closed-cycle helium cryocooler. A pulsed solenoid valve introduced a ~1 ms burst of helium buffer gas, seeded with 10% D_2, into the trap volume, which thermalized the trapped ions and formed weakly bound D_2-tagged adducts. To acquire the one-laser IR predissociation spectrum, the $Gly_3H^+(D_2)$ complexes were ejected into the time-of-flight (TOF) mass spectrometer, mass selected via a gated deflector, and intersected with the output of a Nd:YAG pumped tunable OPO/OPA laser. Resonant absorption of a single photon was sufficient to induce the dissociation of the D_2 tag. The Gly_3H^+ photofragments were separated from $Gly_3H^+(D_2)$ in a two-stage reflectron, and photofragment intensity was monitored as a function of photon wavelength to yield the IR spectrum. The intensities in each spectral region, i.e. the 1000–2300 cm^{-1} and 2800–3800 cm^{-1} regions, are normalized to the most intense feature in the respective region.

To acquire isomer-specific IR-IR ion-dip spectra, detailed in Ref. [28], the output of another Nd:YAG pumped tunable OPO/OPA laser, i.e. the "pump laser", intersected the trapped ions at the center of the 10 K 3D ion trap. The pump laser was triggered ~90 ms after the introduction of the buffer gas to ensure no new tagged adducts could form after photofragmentation, and all trapped ions were ejected into the TOF region ~5 ms after the pump laser. In these experiments, the "probe laser", i.e. the laser intersecting the D_2-tagged ions inside the TOF region, was fixed at a frequency resonant with a specific vibration while the pump laser was scanned. When the pump laser frequency was resonant with a vibration belonging to the same structure as the probed transition, the depletion of that structure inside the ion trap led to a dip in the probed photofragment signal. This ion-dip as a function of pump laser frequency yielded the isomer specific spectra.

The ten lowest-energy structures found by Mookherjee et al. [4] via an extensive search, which also included structures found by Wu and McMahon [1] and Li et al. [30], were re-optimized with various DFT methods and MP2 using several different basis sets. All calculations were carried out using Gaussian 16 [31]. The structures and energies of these ten structures are summarized in Fig. S1 and Table S1 in the Supplementary information. For clarity, we will name them according to the protonation site and configuration of the amide groups. The O^t and N^t isomers have protonation at the amide (C=O^1) or amine (N^1) site, respectively, with both amides in *trans* configuration, while the N^c isomers are protonated at N^1 and have one amide in *cis* configuration. Within each series, the structures are alphabetized according to their relative energies. All the DFT calculations resulted in O^tA as the lowest energy isomer, while MP2 predicts N^cA as the lowest in energy. Despite this disagreement, the various levels of theory point to the same six structures as the lowest energy isomers (see Fig. 2 and Table 1). We will focus on these structures as we make spectral assignments.

Based on the general good agreement in the cam-B3LYP/def2TZVPP harmonic spectra (see Figs. S2 and S3) they are used for comparison with experimental IR spectra in the following discussions. Calculations further show that the D_2 tag has only minor perturbations on the Gly_3H^+ vibrational spectrum, as shown in Figs. S4 and S5. For simplicity, the bare Gly_3H^+ harmonic spectra are used in our analyses. To scale the harmonic spectra, we compared the calculated frequencies of the O^tA isomer carboxyl group OH and CO stretches to those found experimentally. For the cam-B3LYP/def2TZVPP harmonic spectra, this yielded a scaling factor of 0.968 in the 1000–2300 cm^{-1} range and 0.947 in the 2800–3800 cm^{-1} range.

3. Results

The one-laser IR predissociation spectrum of $Gly_3H^+\cdot(D_2)$ is shown in Fig. 3A. It contains spectral features relating to the O—H and N—H stretches (2800–3600 cm^{-1}), the amide I modes (mostly C=O stretches, 1600–1800 cm^{-1}), and the amide II modes (combination of C=N stretches and N—H bends, 1500–1600 cm^{-1}). Additionally, there is an extremely broad feature that extends from ~1300 cm^{-1} all the way to ~2200 cm^{-1}, and is most noticeable by the sloping baseline in the 1800–2200 cm^{-1} region. In the 3000–3600 cm^{-1} region, a single Gly_3H^+ structure is expected to have up to six distinct features arising from the six O—H and N—H bonds present in the molecule. Fig. 3A shows nine distinct features in that region. Furthermore, it also shows five overlapping and partially resolved peaks in the 1600–1800 cm^{-1} region, where only three transitions, corresponding to the three C=O moieties, are expected. These observations suggest multiple structures of Gly_3H^+ are contributing to the one-laser spectrum, similar to the IRMPD studies [1,2].

The two isomer-specific IR-IR spectra, shown in Fig. 3B and C, were acquired with the probe laser wavelength fixed at 3480 cm^{-1} and 3200 cm^{-1}, respectively. Spectrum B has six N—H and O—H stretch features at 3035 cm^{-1}, 3246 cm^{-1}, 3347 cm^{-1}, 3438 cm^{-1}, 3481 cm^{-1}, and 3578 cm^{-1}. The 3035 cm^{-1} feature is significantly broader than the others, indicating that it is associated with an N—H or O—H that is involved in a strong hydrogen-bond. The amide I region shows three narrow features at 1698 cm^{-1}, 1746 cm^{-1} and 1770 cm^{-1}, consistent with the dominant presence of a single isomer. Spectrum C is quite distinctive from spectrum B. The N—H and O—H stretch region has five peaks at 3205 cm^{-1}, 3358 cm^{-1}, 3397 cm^{-1}, 3457 cm^{-1}, 3572 cm^{-1}. Again, the lowest frequency feature is broader than the others, although it is 170 cm^{-1} bluer than the similar feature in spectrum B. The C=O stretch region is dominated by two features at 1684 cm^{-1} and 1790 cm^{-1}, which are both surprisingly broad. Additionally, spectrum C contains the very broad feature that was noted earlier in the