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Vibrational and photoionization spectroscopy of biomolecules: Aliphatic amino acid structures

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The aliphatic amino acids glycine, valine, leucine, and isoleucine are thermally placed into the gas phase and expanded into a vacuum system for access by time of flight mass spectroscopy and infrared (IR) spectroscopy in the energy range of 2500–4000 cm^{-1} (CH, NH, OH, and stretching vibrations). The isolated neutral amino acids are ionized by a single photon of 10.5 eV energy (118 nm), which exceeds by less than 2 eV their reported ionization thresholds. As has been reported for many hydrogen bonded acid-base systems (e.g., water, ammonia, alcohol, acid clusters, and acid molecules), the amino acids undergo a structural rearrangement in the ion state (e.g., in simplest form, a proton transfer) that imparts sufficient excess vibrational energy to the ion to completely fragment it. No parent ions are observed. If the neutral ground state amino acids are exposed to IR radiation prior to ionization, an IR spectrum of the individual isomers for each amino acid can be determined by observation of the ion intensity of the different fragment mass channels. Both the IR spectrum and fragmentation patterns for individual isomers can be qualitatively identified and related to a particular isomer in each instance. Thus, each fragment ion detected presents an IR spectrum of its particular parent amino acid isomer. In some instances, the absorption of IR radiation by the neutral amino acid parent isomer increases a particular fragmentation mass channel intensity, while other fragmentation mass channel intensities decrease. This phenomenon can be rationalized by considering that with added energy in the molecule, the fragmentation channel populations can be modulated by the added vibrational energy in the rearranged ions. This observation also suggests that the IR absorption does not induce isomerization in the ground electronic state of these amino acids. These data are consistent with theoretical predictions for isolated amino acid secondary structures and can be related to previous IR spectra of amino acid conformers. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2902980]

I. INTRODUCTION

Modern molecular biology teaches that molecular structure (e.g., DNA, RNA, proteins, carbohydrates, neurotransmitters, etc.) is an essential component of proper biological function. Just what intra- and intermolecular interactions control these higher order structures, beyond simple chemical connectivity, becomes a topic of some interest and considerable research.[1–9] Certainly, water intermolecular interactions are known to be a major factor controlling and/or assisting the generation of these higher order structures through both enthalpic and entropic influences. Additionally, the energy minimum isomeric structures of individual molecules, such as amino acids and sugars, are required to generate a functional understanding of how their polymers achieve their eventual solution phase, "folded biologically active" structures. The process of unraveling the mechanisms and essential features of the final structures for proteins and carbohydrates, for example, is thus founded on an understanding of isolated amino acid, sugar, peptide, saccharide, etc., structures. The structures of these individual building blocks of biopolymers can be accessed in two ways: gas phase studies of these amino acids, sugars, neutrotransmitters, and model systems, and theoretical quantum mechanical potential energy surface calculations for these building blocks. Clearly, both approaches are required for proper data interpretation and to prove theoretical models.

In this report, we discuss the IR spectra and single photon, ionization/fragmentation spectra of four aliphatic amino acids: Glycine, valine, leucine, and isoleucine. Amino acids have the general formula RCH(NH_2)COOH, R=H, $(CH_3)_2$CH, $(CH_3)_2$CHCH_2, and CH_3CH_2CH(CH_3) for glycine, valine, leucine, and isoleucine, respectively. Each of these amino acids has three major structural isomers involving the CH(NH_2)COOH moiety hydrogen bonding patterns, as predicted by theory.[1,10,11] These general structures are such that in all instances, the O—H moiety is hydrogen bonded (to either the NH_2 or CO group) and additionally zero, one, or two of the NH_2 hydrogens are involved in hydrogen bonding with the COH or CO oxygen atom. These structures are illustrated in Fig. 1.[11] Of course, other isomers exist that are of much higher energies than the three presented in Fig. 1; however, all theoretical studies, while not always agreeing on the ordering of these conformer energies,

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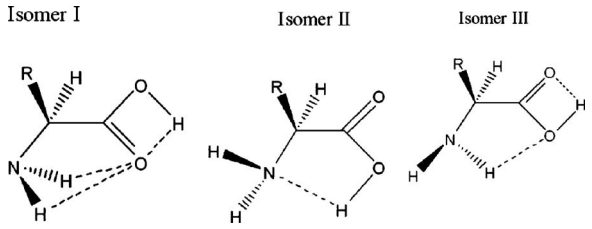


FIG. 1. Calculated structures for general amino acid isomers according to Ref. 11.

obtain these three structures as lower in energy than others. Since the temperatures employed in our studies do not exceed 200 °C and the samples are cooled in a supersonic He expansion, only these low lying conformers of the amino acids studied should be considered. Barriers to their interconversion in their ground states can be as high as hydrogen bonding energies (~5–10 kcal/mol or 0.44 eV, see Ref. 6(b) for a determination of dipeptide barriers).

Isomer I has all the labile hydrogens of the general amino acid *R*CH(NH_2)COOH involved in hydrogen bonding, isomer II has both of the NH_2 group hydrogens free from hydrogen bonding but the OH group hydrogen bonded to the NH_2 lone pair electrons, and isomer III has only one of the NH_2 hydrogens bonded to the COH oxygen moiety. For isomer III, the OH group hydrogen is additionally hydrogen bonded to the CO oxygen moiety.[10] The calculated energies of the three isomers in simple nonaromatic amino acids are generally $E(\mathrm{I}) < E(\mathrm{II}) \leqslant E(\mathrm{III})$.[11] The relative energies of these structures are most likely correctly estimated but their numerical differences are probably not accurately calculated. Many more conformers should exist for each of these major isomers, as the orientational preferences of the *R* group within itself and with respect to the rest of the amino acid framework are expressed.[11] In the present study, we assume that these additional conformational isomers of the above four amino acids are not resolved by our spectroscopic measurements.

As one can imagine, the three isomers depicted in Fig. 1 should have very different spectra in the 2500–4000 cm^{-1} spectral range, in which CH, NH_2, and OH stretching modes will absorb. According to the classification of hydrogen bonds, the above NH_2—OH bonding structures can be considered to be what Kozich *et al.*[12] considered as "medium-strong" hydrogen bonds. Thereby, one can expect these vibrational modes for the hydrogen bonding isomeric structures to be shifted and broadened in energy for N—H···O, N···H—O, and O···H—O structures to ~3000 cm^{-1}, which are roughly degenerate with the CH modes. Kozich *et al.*[12] show, through cw and time resolved measurements, that these hydrogen bonded mode structures are quite complicated and thoroughly mixed, through Fermi resonance and anharmonicity, with each other and lower energy bending modes of all three groups (CH, OH, and NH), as well as others. These findings have been recently corroborated for amino acids by a study of arginine under various conditions of solvation and ionic states.[13]

The general study of hydrogen bonding within solutions has been carried out by Kozich *et al.*[12] to determine widths, shifts, couplings, and lifetimes for intra- and intermolecular hydrogen bonding. Their results are well summarized in a recent review article[14] and briefly mentioned above. Gas phase studies of amino acids and peptide systems have been reported by Ryan *et al.*,[2] Chin *et al.*,[15] Kleinermanns and co-workers,[16] Abo-Riziq *et al.*,[17] Lee *et al.*,[18] Unterberg *et al.*,[19] Alonso and co-workers,[3,11] Simons and co-workers,[20] and others,[21–23] including theory, spectroscopy, and ionization studies. For the studies involving IR/UV double resonance spectroscopy, in which the CH, NH, and OH vibrational modes are accessed and analyzed, the reported studies involve amino acids that contain an aromatic moiety. The aromatic ring (phenyl or indole) serves as a chromophore through which two photon resonance ionization can be achieved in order to detect the vibrational mode excitation in the electronic ground state. The ion state is then localized on the aromatic moiety and is relatively weakly coupled the (NH_2)CHCOOH amino acid substituent. Analysis of these spectra is generated typically through density functional theory (DFT) calculations of the anticipated vibrational modes for different conformers with regard to the overall amino acid structure and hydrogen bonding patterns. OH, NH, and CH modes are characterized and are typically determined to shift to lower energy (a few tens to hundreds of cm^{-1}) due to hydrogen bonding. These reported shifts and widths are smaller than those found by Kozich *et al.*, which reports OH and NH modes to move into the CH mode region at ~2800–3200 cm^{-1}. All three hydrogen stretch modes then couple and become thoroughly mixed with one another and lower energy mode overtones and combinations. The spectra obtained in the present studies are best interpreted in terms of the findings of Kozich *et al.* One possible reason for such differences between these very small amino acids (glycine, valine, leucine, and isoleucine) and the larger aromatic species and peptides and model peptides[15] may be that the smaller species are more strongly hydrogen bonded (perhaps with the exception of glycine, which may be too small to form strong hydrogen bonds). These issues will be discussed further below.

Ionization and ion reaction studies of simple amino acids have also been presented based on both calculations and simulations and on experiments.[24] Proton transfer, structural rearrangements, and fragmentation reactions have been characterized. Absorption of a photon to generate the Franck–Condon structure for the ground state ion typically leads to a reaction that drives the ion fragmentation. Such studies have been reported for a number of simple aliphatic amino acids including glycine, valine, leucine, and isoleucine. The reported fragmentation patterns observed for these systems are essentially the same as reported in the present study, considering the different ionization energies employed.[24(a)] The essential point here is that ionization engenders a rearrangement reaction, the enthalpy of which opens fragmentation channels for the photon created, newly ionized, ground electronic state ion. No studies of simple amino acid mass spectroscopy find significant parent ion signals including the present one.[24] The idea of a structural rearrangement reaction which imparts vibrational energy into the molecule following its ionization is quite typical for hydrogen bonded systems in

general. Such reactions follow ionization for many van der Waals systems and clusters including naphthol $(NH_3)_n$, $n \geq 3$,[25(a),25(b)] $(NH_3)_n$,[25(c)] $(H_2O)_n$,[25(c)] $(HCOOH)_n$,[25(d)] $(CH_3COOH)_n$,[25(e)] (CH_3CH_2COOH),[25(f)] $(CH_3OH)_n$,[25(g),25(h)] $(C_2H_5OH)_n$,[25(i)] and others. This will be discussed further below, as well.

The vibrational spectra of general amino acids, especially in the 2500–4000 cm^{-1} near infrared (IR) region, are quite sensitive to environmental perturbations, particularly those that change the higher order structures of the species under investigation. The reason for this is that the intramolecular interactions that tend to control and shape fundamental isomeric structures for amino acids are of the order of 5–10 kcal/mol, and the intermolecular interactions with solvents such as He, Ar, hydrocarbons, etc., are also of the same magnitude. Thus, vibrational spectra of aromatic substituted amino acids or amino acids in "inert" solvents such as Ar matrices, hydrocarbons, water, or other species may not be qualitatively identical to the vibrational spectra of these isolated gas phase amino acids.[1(c)–1(e),26] Additionally, Linder *et al.* have presented the first gas phase Fourier transform infrared (FTIR) absorption spectra in the range of 900–3700 cm^{-1} [Ref. 16(b)] of neutral amino acids without aromatic moieties by using a fast thermal heating technique. In these studies, the molecules are hot in the gas phase and show no hydrogen bonding or specific isomers. Thus, we can compare our studies with others, but we can anticipate that not all IR spectra of the accessed matrix isolated amino acids will yield identical results to those presented here for glycine, valine, leucine, or isoleucine.[1,27] These comparisons will be made as our data are discussed in Sec. IV below.

The studies presented in this report are for the simplest (nonpolar), aliphatic amino acids that do not have an aromatic moiety as an ionization chromophore. The ionization/detection step for the IR vibrational absorption in our experiments is through a single photon of 118 nm wavelength or 10.5 eV energy. This energy is sufficient to directly ionize all amino acids. Clearly, the advantage of this approach is that any biological molecule (neurotransmitter, amino acid, sugar, etc.) can be available for IR/UV double resonance or ion dip experiments and can thereby have its structure studied through the analysis of its CH, NH, OH, and other vibrational modes. The ion in the present case is generated from the lone pair nitrogen orbital (or possibly a mixed N—O orbital[4]), which is the highest occupied molecular orbital (HOMO), and thus is directly involved with the amino acid (NH_2)CHCOOH molecular moiety. This leads to a structural rearrangement upon ionization (possibly more complex than a simple proton transfer reaction) and the energy that is released into the vibrational degrees of freedom of the ions (ΔH_{react}) by this reaction can cause the ions to fragment. This initial complication for IR/UV double resonance detection (no parent ion signal) also has an advantage, as the fragmentation patterns for the ionized amino acids turn out to be conformer dependent, as are the IR absorption spectra. Thus, one can suggest which IR spectrum is associated with a particular isomer (in general, I, II, or III, Fig. 1) and can suggest which fragment is associated with a particular isomer. Qualitative agreement for both of these associations generates confidence in both the theory and the analysis that leads to such assignments.

In this report, we discuss the IR absorption spectra and fragmentation of the aliphatic amino acids glycine, valine, leucine, and isoleucine detected as described above through IR absorption and single photon ionization. We find IR spectra consistent with theoretically predicted low energy isomers of these amino acids and with anticipated fragmentation patterns for the respective isomeric ions.

II. EXPERIMENTAL PROCEDURES

The experimental apparatus used to record time of flight mass and IR spectra in combination with a vuv single photon ionization source has been previously described in detail.[28] Commercial samples of amino acids (Aldrich) are used without further purification. The solid sample is placed close to the valve body (Parker General Valve series 9) and is heated to increase its vapor pressure. The entire nozzle body is heated to the sample temperature, which is typically 150–200 °C. This provides an adequate stream of target molecules, with sufficiently low temperature to ensure that the thermally fragile low volatility amino acids remain undissociated in the gas phase.[24(c)] The gaseous amino acid molecules are seeded into an argon/helium gas mixture (70%/30%, total pressure of 2 atm) and the gaseous mixture is expanded into a high vacuum chamber by the pulsed supersonic nozzle with a pulse width of typically 150 μs duration. After passing through a skimmer, the molecular beam interacts with pulsed vuv and IR laser beams in the ionization region of a time of flight mass spectrometer (TOFMS), in which vuv laser generated ions are detected.

An IR laser beam, which counterpropagates with respect to the 118 nm laser beam, is focused at the vuv/molecular beam intersection point by a 40 cm focal length lens to access neutral ground state species. Generation of the vuv 118 nm light and IR laser light is similar to that described earlier.[25(i),28] The 118 nm radiation is the ninth harmonic of the fundamental output of a Nd^{+3}/yttrium garnet laser at 1.064 μm. 355 nm radiation (third harmonic) is focused into a cell with Xe/Ar at a ratio of 1:10 at ~200 Torr total pressure. A MgF_2 lens focuses the 118 nm light in the ionization region of the TOFMS and disperses the remaining 355 nm light. Further separation of the 355/118 nm radiation can be achieved by inserting a MgF_2 prism into the beam. Such separation, however, has no influence on the observed mass spectra. Tunable IR light output in the range of the mid-IR and near IR has a pulse energy of 3–5 mJ/pulse and a bandwidth of ~2 cm^{-1}.

The IR absorption spectra are measured by the use of IR plus vuv nonresonant ionization and fragmentation detection spectroscopy, which has been previously employed for the study of alcohol and organic acid hydrogen bonded clusters.[25(c)–25(i)] The principle of IR plus vuv nonresonant ionization and fragmentation detection of the vibrational spectra of amino acid molecules is similar to that reported for ethanol and ethanol clusters. Note that the vuv nonresonant photon energy (10.49 eV) is close to the ionization en-

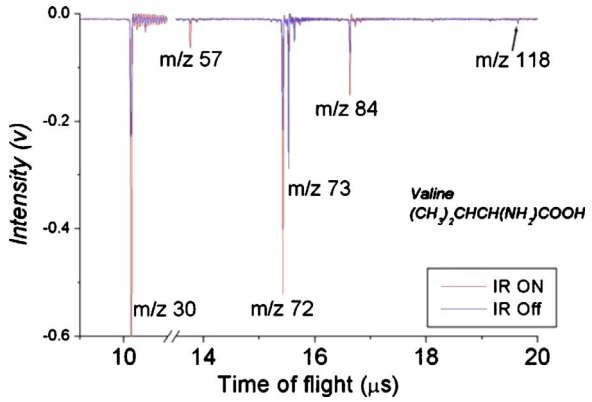


FIG. 2. (Color online) Mass spectra for valine ionized with 118 nm light with the IR laser (~3000 cm^{-1}) on (red) and off (blue). Note that most mass channels increase in intensity with the IR laser on but mass channel 73 amu decreases in intensity with the IR laser on. The weak feature located at m/z 118 in the mass spectrum is assigned as the protonated valine cation, which arises from the photodissociation of the valine dimer. The gap between mass ~31 and mass 57 amu does not contain features.

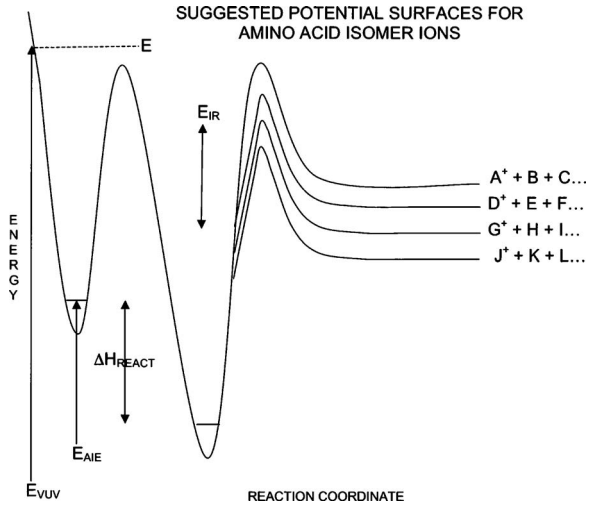


FIG. 3. Schematic drawing of the potential energy surfaces of the amino acid ions for a given isomer structure. Since the ionization process occurs in attoseconds and the nuclei move in femtoseconds, the vertical ionization position is the same as the ground state equilibrium position for a given conformer. This energy is apparently sufficient, in terms of excess vibrational energy in the amino acid $[(E_{ion}(vib))=(E_{VIE}-E_{AIE})]$, to surmount a barrier to a rearrangement reaction in the ion. The ΔH for this reaction then adds to the ion vibrational energy as does the vibrational excitation (IR absorption) added to the neutral amino acid in the ground state absorption experiment. This total vibrational energy in the ion can influence the population of the various fragmentation channels open to the ion. Note that these channels are not changed by the vibrational energy absorption. Nonetheless, the existing fragmentation channel can change population (positive and/or negative) due to the absorption of CH, NH, or OH vibrational energy. All the *X*H modes change the fragment intensity the same way, positive or negative.

ergy (IE=8.0–10.5 eV) [Ref. 24(c)] of the aliphatic amino acids. As the IR laser is scanned to excite cooled molecules to higher vibrational modes of their ground electronic state prior to the introduction of vuv light, the cation or fragment ion mass channel intensity is monitored. The vibrational spectrum of the neutral isomeric molecules is thereby obtained, as the fragmentation pattern depends on the total energy in the amino acid cation and on the isomeric structure of the amino acid. Both positive and negative intensity changes in the fragment mass channels can be observed as a function of IR absorption, as the various channels for fragmentation become more or less accessible depending on the total energy in the amino acid parent ion.

III. RESULTS AND DISCUSSION

Consider first the mass spectrum of valine, as presented in Fig. 2. This figure has two mass spectra superimposed: The blue line represents the mass spectrum of valine, generated with 118 nm ionization and the red line represents the mass spectrum of valine obtained with both IR (~3000 cm^{-1}) and 118 nm radiations. The IR probe (~10 ns) precedes the 118 nm pulse (~10 ns) by ~30–50 ns. As can be seen in the figure, the signals in mass channels 30, 57, 72, and 84 amu all increase in intensity with the addition of IR radiation (~3000 cm^{-1}), while the signal in mass channel 73 amu decreases in intensity with the addition of IR radiation. Clearly, the IR radiation does not cause the fragmentation of valine (117 amu), as new mass channels do not readily appear with its presence. Moreover, this is not the experience for other IR/UV studies of amino acids with aromatic chromophores.[5,6] Such extensive fragmentation is not reasonable based on at most ~1.5 eV (12 000 cm^{-1}) of excess energy in the valine ion.

Thus, the energy required for the observed fragmentation most likely arises from a structural rearrangement of the initially created ion; such a rearrangement reaction in its simplest form could be a proton transfer reaction, but in this instance the rearrangement is most likely more complicated and severe. This reaction is not necessarily specifically isomer dependent but it must lead to different fragmentation channels for the different isomers. A proton transfer reaction in such an ion could provide as much excess energy (proton affinity) to the newly formed ion as 200 kcal/mol (~9 eV).[29] This ΔH_{react} would then be added to the unfragmented ion in the form of vibrational energy. In all instances, we assume that intramolecular vibrational redistribution (IVR) of energy is fast ($\lesssim 10^{-12}$ s) and especially faster than vibration predissociation (VP). VP should be relatively slow ($\gtrsim 10^{12}$ s) for these large molecules at such high vibrational excitation and density of states.[29] Such behavior is quite typical for van der Waals clusters of $(H_2O)_n$, $(NH_3)_n$, $(ROH)_n$, $(RCOOH)_n$, naphthol $(NH_3)_n$, and others.[25,29] Thus, as illustrated in Fig. 3, the mechanism for the observed fragmentation of valine upon ionization at 10.5 eV is possibly as follows: The ion is formed with enough excess energy to surmount any barrier to the overall structural rearrangement to a new lower energy geometry; the ΔH_{react} for this rearrangement is generated in the intact ion (for a given isomer) and contributes to the ion vibrational energy $[E_{vib}(ion)=(E_{VIE}-E_{AIE})+\Delta H_{react}]$; this rearrangement provides enough energy to surmount the barriers to molecular fragmentation of the ion; the lowest barrier for the reaction would then be for the m/z species at 73 amu, with 72, 30, 84, and 57 amu species as the apparent ordered set of higher barrier mass channels for fragmentation (see Fig. 3). Absorption of IR radiation allows these higher barrier channels to become more populated and thus gain intensity at the expense of

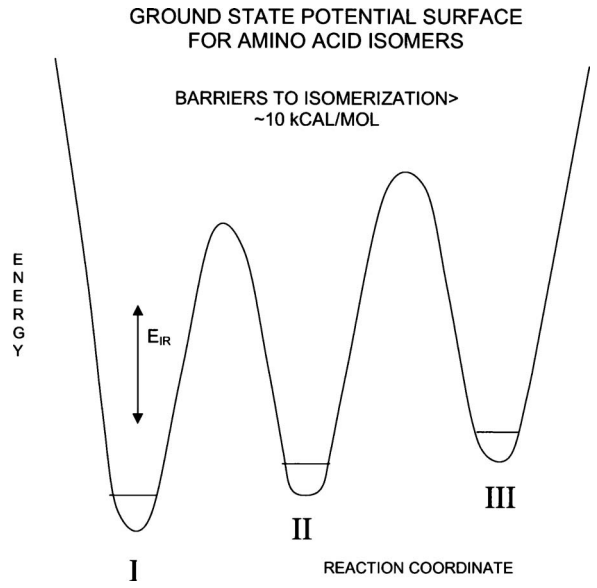


FIG. 4. Schematic drawing of the ground structure state potential energy surface for the three lowest energy isomers for each amino acid. The barriers and IR energies are roughly to scale for the hydrogen bonding energies. The energy differences between the respective minima are of the order of a few kcal/mol and are calculational algorithm and basis set dependent (see text for further discussion of these points).

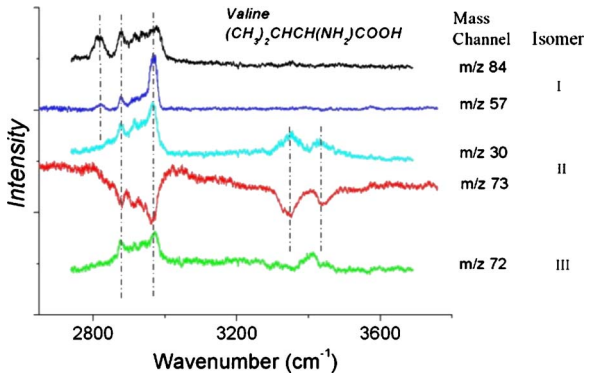


FIG. 5. (Color online) The IR spectra for valine isomers observed in different fragment mass channels as indicated. The isomers identified in the figure are suggested based on the structures given in Fig. 1, the fragmentation mass channels, and the IR spectra.

mass channel 73 amu intensity or population. Note that all isomers need not show a loss of intensity for a given mass channel, as such behavior must depend on the details of the rearranged ion potential energy surface and the values of ΔH_{react} and $(E_{\text{VIE}}-E_{\text{AIE}})$ for a given isomeric form. Below, we show that all these fragments are not from a single isomer and thus the above generalization can be somewhat modified.

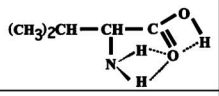
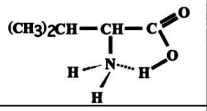
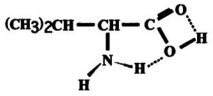
More than one IR photon could be absorbed in this process, but not sufficient numbers to cause IR multiphoton ionization (MPI) or to change fragmentation patterns. Additionally, as we will discuss below, IRMPI should irradicate any subtle fragmentation differences between isomers in the fragmentation reaction chain, as IRMPI on the nanosecond time frame is known to generally heat molecules in an incoherent fashion, which should remove any isomeric reaction preferences [see Ref. 16(b), in particular].

One might suggest that the IR absorption would change the isomer distribution in the ground state (see Fig. 4). If this were the case, more than one fragment ion channel would evidence a "negative" intensity change upon IR irradiation of the sample (see below). Since this is not the case, ion fragmentation channels must populate and depopulate with the total vibrational energy in the rearranged molecular ion for each isomer separately. Note too that IR/vuv irradiation occurs under isolated molecule conditions in the beam. Thus, energy to surmount the barriers between conformations in the ground state will remain in the molecule and isomer distinction will not be maintained. Isomer specific amino acid fragmentation behavior should not be expected for this mechanism.

The mass spectra of valine obtained with ionization through 10.5 eV single photons (with and without IR excitation preceding ionization) is mechanistically informative and leads to the suggestion that the amino acid fragments due to a rearrangement reaction following the ionization step. The reaction induced fragmentation occurs through a number of channels whose barriers can be surmounted in many instances (but not necessarily all) by $\sim$3000 cm^{-1} of excess energy in the CH, NH, and/or OH stretching modes. We do not suggest that this behavior is at all nonstatistical (i.e., IVR is much faster than VP) and we believe that if the details of the accurate potential energy surface for the ions were available, an RRKM calculation for the unimolecular fragmentation reactions would qualitatively explain the results.[29(a)] Additionally, the mass spectra of valine shown in Fig. 2 are taken for a more concentrated sample (the vapor pressure of the valine will be up to 2.0 torr at $\sim$200 °C [Ref. 24(h)] than those of the other amino acids such as glycine, leucine, and isoleucine, and valine clusters can thereby form in the expansion. Thus, protonated valine cations (m/z 118) are present in the mass spectrum. This feature, as well as others at higher mass, arises due to single photon ionization and fragmentation of valine dimers in the expansion. These spectra reflect the scope of future studies for clusters of amino acids and how they respond to IR radiation and 118 nm single photon ionization. In this study, the concentration of valine vapor in the expansion gas is controlled by maintaining the nozzle body and sample temperature below 200 °C, in order to avoid formation of clusters in the beam. The small feature for protonated valine in the mass spectrum shows that cluster formation is depressed in this experiment. Thus, the obtained IR spectra are indicative of isolated monomers.

Figure 5 shows the IR spectrum obtained in these mass channels for valine fragments 84, 73, 72, 57, and 30 amu. Note first that three distinct types of spectra are obtained. Mass channels m/z 84 and 57 amu display transitions only in the classical CH region $\sim$2900 cm^{-1}, and the OH ($\sim$3600 cm^{-1}) and NH ($\sim$3400 cm^{-1}) mode absorptions are "missing." Second, the IR spectra detected while monitoring mass channels 73 and 30 amu show two features in the "NH" range $\sim$3400 cm^{-1}. The spectrum monitored in mass channel 73 amu is negative (arises from a loss of intensity in the mass channel), while the IR spectrum obtained by monitoring mass channel 30 amu is positive (arises from a gain in intensity in the mass channel). Third, the IR spectrum obtained by monitoring mass channel 72 amu evidences only

TABLE I. Isomers of valine, possible IR absorption assignments, and ionization and suggested fragmentation processes under IR plus vuv radiation for each of the main lower energy conformers. Note that other rearrangements of fragments are possible.

Isomer Structures	Ionization and Fragmentation Process	Energies (cm^{-1}) and Vibrational Modes
I 	Cations m/z 84 $(CH_3)_2CHCH(NH_2)COOH \xrightarrow{h\nu} NH_2OH + (CH_3)_2CHCHCO^+$ Cations m/z 57 $(CH_3)_2CHCH(NH_2)COOH \xrightarrow{h\nu} (CH_3)_2CH^\bullet + OH^\bullet + NH_2CHCO^+$ $\xrightarrow{h\nu} (CH_3)_2CH^\bullet + NH_2^\bullet + CHCOO^+$	2800~3000, CH, OH, NH stretches
II 	Cations m/z 73 $(CH_3)_2CHCH(NH_2)COOH \xrightarrow{h\nu} CO_2 + (CH_3)_2CHCHNH_3^+$ Cations m/z 30 $(CH_3)_2CHCH(NH_2)COOH \xrightarrow{h\nu} (CH_3)_2CH^\bullet + CO_2 + CH_4N^+ (CHNH_3^+)$	2800~3000, CH, OH stretches 3346, Symmetric NH stretch 3436, Asymmetric NH stretch
III 	Cations m/z 72 $(CH_3)_2CHCH(NH_2)COOH \xrightarrow{h\nu} COOH^\bullet + (CH_3)_2CHCHNH_2^+$	2800~3000, CH, OH stretches 3409, NH stretch

one absorption feature in the NH region (~3400 cm^{-1}) in addition to the multiple absorption features in the classical CH mode region near ~2900 cm^{-1}.

Based on the structures presented in Fig. 1 and the background understanding of the perturbation of NH and OH modes in the presence of hydrogen bonding interactions,[1,12–14,27,30] we suggest that the spectra detected by monitoring mass channels 57 and 84 amu belong to isomer I (both NH_2 and OH moieties are involved in hydrogen bonding), the spectra detected in mass channels 73 and 30 amu belong to isomer II (only the OH moiety is involved in hydrogen bonding), and the spectrum detected by monitoring mass channel 72 amu belongs to isomer III (the OH moiety and one of the NH hydrogens are involved in hydrogen bonding). If the ground state isomer interconversion mechanism were operative here, we would expect to find both mass channels 30 and 73 amu with negative intensity change upon IR irradiation as isomer II would depopulate to yield isomer I and/or III. Moreover, if the fragmentation reaction were to occur on the ground state potential energy surface, new ions would be observed in the mass spectra with the addition of IR radiation energy: Some of the observed fragments could not be ionized by 118 nm radiation, while others could be ionized (e.g., COOH, C_2H_3O, C_3H_7, etc.)

The reports of Elsaesser and co-workers[12,30] on NH and OH hydrogen bonding suggest that the hydrogen bonded NH and OH stretching modes are broadened, mixed with other modes and combination and overtone modes, and shifted in energy to the ~3000 cm^{-1} region to mix with the CH modes. This highly anharmonic perturbation probably also explains the apparent intensity of the CH mode, most likely at ~3000 cm^{-1}. The observed absorption is due to the stretching modes, CH, OH, and NH, not observed at higher energy, in addition to lower energy modes whose overtones and combinations can also appear in this energy range.[12,30] In any event, the OH and CH modes seem to be overlapped for all three isomers, and the NH modes may or may not be observed separately depending on the monitored isomer fragment.

Table I shows a suggestion for the ions observed in the different fragment mass channels, their associations with different isomers, and the observed vibrational modes and energies. Note that the proposed fragmentations are qualitatively consistent with isomeric structures and with the observed fragment detected IR spectra as analyzed above. Clearly, other fragment channels would be possible; nevertheless, the observed ones can be rationalized with the theoretically predicted isomers and represent minimal structural rearrangements. The observed fragmentation patterns are also consistent with those previously reported.[24] One cannot immediately determine the most abundant structures based on isomer energies because the fragment mass channel intensities are also governed by the fragmentation probabilities, which are at present unknown. Note that the isomers, IR spectra, and fragments comprise an internally consistent set and mutually support the various components of the data analysis.

Mass spectra for glycine (75 amu) with (red) and without (blue) IR excitation are presented in Fig. 6. Signals detected in mass channels 30 and 42 amu are changed in intensity by almost a factor of 2 under IR irradiation of glycine, and mass channels 31 and 43 amu signals are changed by

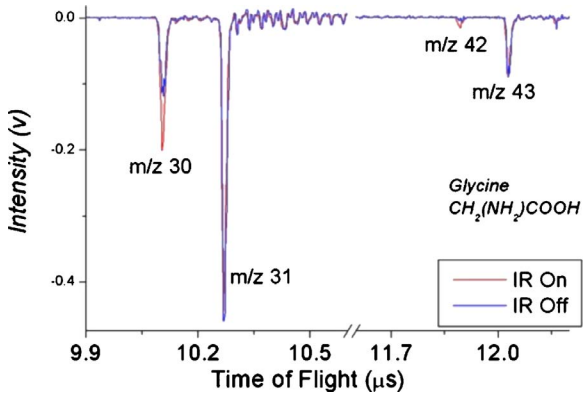


FIG. 6. (Color online) Mass spectra for glycine ionized with 118 nm light with the IR laser (~3000 cm^{-1}) on (red) and off (blue). Note that both mass channel signals 30 and 42 increase with the presence of IR radiation, while mass channels 31 and 43 seem little affected by the presence of IR radiation.

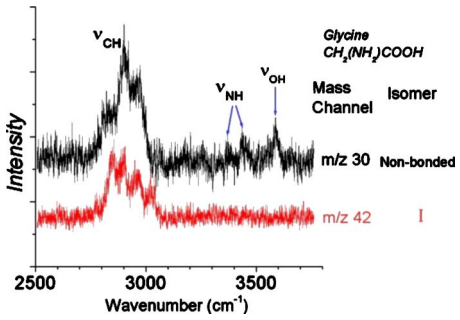


FIG. 7. (Color online) The IR spectra for glycine isomers obtained in different fragment mass channels as indicated. The isomers identified in the figure are suggested based on the structure given in Fig. 1, the fragmentation mass channels, and the IR spectra.

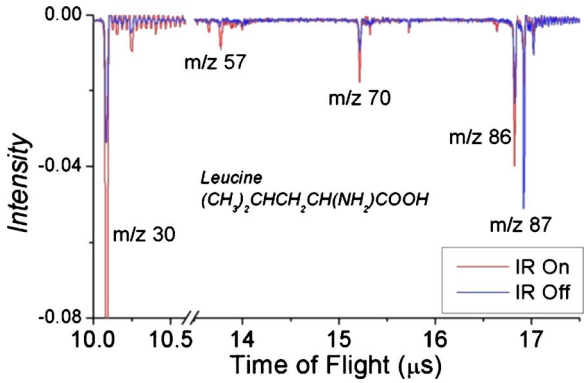


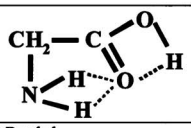
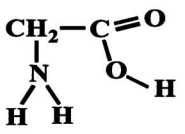
FIG. 8. (Color online) Mass spectra for leucine ionized with 118 nm light with the IR laser (~3000 cm^{-1}) on (red) and off (blue). Note that most mass channels increase in intensity with the IR laser on but mass channel 87 decreases in intensity with the IR laser on.

$\leqslant$10% upon IR irradiation of glycine. This fragmentation pattern is consistent with recent predictions based on DFT calculations.[4] Due to overall weak signals for glycine spectra, in general, only the spectra obtained in mass channels 30 and 42 amu will be discussed here.

Figure 7 shows the IR spectra of glycine isomers, as detected in mass channels 30 and 42 amu. The IR spectrum detected in mass channel 30 is weak and appears to show all three CH, NH_2, and OH characteristic stretching features. The low spectral intensity of glycine here suggests that in agreement with the absence of strong hydrogen bonding for this isomer, the NH_2 and OH modes are not enhanced in intensity and the apparent CH region at ~2900 cm^{-1} is also not enhanced due to the spectral shift and overlap of the latter two modes. The uncrowded nature of the glycine molecule may sufficiently open the geometry of the molecule such that the NH_2, COH, CO, and OH distances are large enough that hydrogen bonding is less strong or less favorable for this case. The "bare" OH and NH_2 stretches appear at ~3587 and 3360 and 3440 cm^{-1}, respectively. On the other hand, the IR spectrum detected in mass channel 42 is clearly consistent with the isomer I structure, as described in the discussion for valine and as shown in Fig. 1. Note that the mass 42 amu species is most likely $C_2H_2O^+$ (ketene), although $C_2H_4N^+$ is also possible. The fragmentation pattern for glycine ($NH_2CH_2^+$ and CH_2CO^+) is consistent with, and supports, the isomer identification for the mass channels observed (see Table II). Note that the CH mode 2800–3100 cm^{-1} region for the two IR spectra obtained for glycine conformers are different in intensity pattern, although somewhat similar in broad features. These differences can be ascribed to the mode coupling associated with the shifting and broadening of OH and NH vibrations for isomer I, which then couple with the CH modes.

The mass spectra for the 118 nm ionization and IR plus 118 nm ionization of leucine (131 amu) are presented in Fig. 8. Mass channels 30, 57, 70, and 86 amu are the prominent ones that increase in intensity with the addition of IR radiation prior to the 118 nm ionization pulse (red spectrum); mass channel 87 amu, on the other hand, decreases in intensity as the IR radiation is scanned. Note that this general mass spectral pattern of fragmentation is not unanticipated.[4,24] One can see this behavior in the IR spectrum of leucine isomers in the different mass channels (Fig. 9). The spectrum detected in mass channel 87 amu is negative and one can observe a decreasing base line beginning near 3500 cm^{-1} and continuing past 3000 cm^{-1} that can be associated with the broadened and shifted NH and OH modes. Similar behavior and IR spectra are identified for mass channels 70, 57, and 30 amu; all four of these mass channels are thereby suggested to arise from fragmentation of conformer I. The fragment mass channels 87 and 30 amu could arise from a modified (I*) isomer I. This suggests that the structure need not be a full zwitterionic form of isomer

TABLE II. Isomers of glycine, possible IR absorption assignments, and ionization and suggested fragmentation processes under IR plus vuv radiation for each of the main lower energy conformers. Note that other rearrangements of fragments are possible.

Isomer Structures	Ionization and Fragmentation Process	Energies (cm^{-1}) and Vibrational Modes
I 	Cations m/z 42 $CH_2(NH_2)COOH \longrightarrow H_2NOH + CH_2CO^+$ $\longrightarrow OOH^\bullet + C_2NH_4^+$	2800~3000, CH, OH, NH stretches
Not H-Bonded 	Cations m/z 30 $CH_2(NH_2)COOH \longrightarrow COOH^\bullet + CH_4N^+$	3587, OH stretch 3360, NH symmetric stretch 3440, NH asymmetric stretch

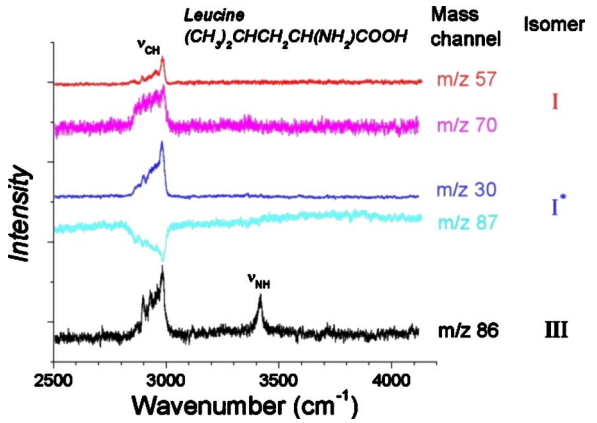


FIG. 9. (Color online) The IR spectra for leucine obtained in different fragment mass channels as indicated. The isomers identified in the figure are suggested based on the structures given in Fig. 1, the fragmentation mass channels, and the IR absorption spectra.

I.[31] It is only suggested based on the observed fragment pattern as given in Table III. The mass 30 amu fragment could also be the formaldehyde ion (H_2CO^+) but this would imply an extreme rearrangement pattern for isomer I. The suggested I* possible structure is consistent with the observed fragmentation pattern. An additional possibility for this fragmentation pattern (through mass channels 87 and 30 amu) would be that the ion rearranges to generate a weakly zwitterionic form that then fragments as indicated. Again, the decreased intensity of the ion signal in mass channel 87 amu is caused by the enhancement of other mass channels as IR absorption occurs for isomer I. This would suggest that the 87 amu fragment channel is the lowest energy fragmentation channel for isomer I, which loses intensity (to the other fragmentation channels) as more energy is placed into the amino acid rearranged ion. Isomer III with one NH hydrogen free from hydrogen bonding shows one NH mode at ~3420 cm^{-1} in the IR spectrum associated with mass channel 86. Similar spectra, both mass and IR, are observed for isoleucine, as can be anticipated. The observed 118 nm mass spectrum for the isoleucine sample employed also shows features associated with isoleucine clusters.

The 2800–3100 cm^{-1} region of the IR spectra displayed above can vary from isomer to isomer and also in some instances even within an assigned isomer set of fragments. The reason for different isomers (I, II, and III) to show different spectra in this region is that the various isomers have different mode couplings and CH anharmonic couplings with different combination and overtone modes (see Refs. 12, 14, and 30). Moreover, the degree of OH, CH, and NH mode mixing is isomer dependent. Additionally, within each isomer, the substituent group R [=H, $(CH_3)_2CH$, $(CH_3)_2CHCH_2$, and $CH_3CH_2CH(CH_3)$] can have different conformations (within itself and with respect to the amino acid moiety, $NH_2CHCOOH$) that can lead to different spectroscopic structures within the heavily mixed mode region ~2800–3100 cm^{-1}.

IV. GENERAL COMMENTS

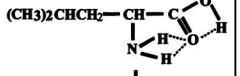
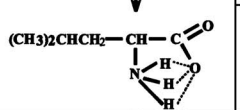
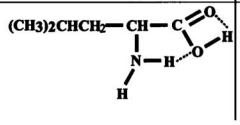
The loss of IR absorption for OH and some NH modes detected in various mass channels arising for particular isomers is more apparent than real because these features are probably perturbed to be broad and shifted to the 3000 cm^{-1} region, at which energy they can couple to the CH modes and enhance their intensity. This has been substantiated by Refs. 12, 14, 26, 27, and 30.

Multiphoton IR absorption while possible is not the main cause for the fragmentation observed because, in general, the mass spectra of the accessed amino acids are identical for 118 nm and IR plus 118 nm excitations. The exception here is the highly concentrated valine and isoleucine samples for which clusters of the amino acids are detected (Fig. 2).

We assume that the IR absorption and subsequent IVR is rapid and that no coherent effects are associated with the observed spectra. This is apparently correct based on Refs. 12, 14, 26, 27, and 30 and the fact that the IR absorption effects on the mass spectra are independent of what modes are accessed.

In all instances, based on our simple *ab initio* calculations, the HOMO is the nitrogen lone pair orbital and the ionized electron is assumed to come from this HOMO.[4] Recent DFT calculations, however, suggest a more mixed O/N lone pair mixture for this orbital.[4]

TABLE III. Isomers of leucine, possible IR absorption assignments, and ionization and suggested fragmentation processes under IR plus vuv radiation for each of the main lower energy isomers. Note that other rearrangements of fragments are possible.

Isomer Structures	Ionization and fragmentation process	Energies (cm^{-1}) and vibrational modes
I 	Cations m/z 70 $(CH_3)_2CHCH_2CH(NH_2)COOH \xrightarrow{h\nu} NH_2COOH\bullet + (CH_3)_2CHCH_2CH^+$ Cations m/z 57 $(CH_3)_2CHCH_2CH(NH_2)COOH \xrightarrow{h\nu} CH(NH_2)COOH\bullet + (CH_3)_2CHCH_2^+$	2800~3000, CH, OH, NH stretches
I* 	Cations m/z 87 $(CH_3)_2CHCH_2CH(NH_2)COOH \xrightarrow{h\nu} CO_2 + (CH_3)_2CHCH_2CHNH_3^+$ Cations m/z 30 $(CH_3)_2CHCH_2CH(NH_2)COOH \xrightarrow{h\nu} (CH_3)_2CH\bullet + CO_2 + CH_4N^+$	
III 	Cations m/z 86 $(CH_3)_2CHCH_2CH(NH_2)COOH \xrightarrow{h\nu} COOH\bullet + (CH_3)_2CHCH_2CH(NH_2)^+$	2800~3000, CH, OH, NH stretches 3421, NH stretch

Finally, the previous studies of IR plus UV ionization spectroscopy of amino acids have been accomplished with aromatic species for which the aromatic moiety carries the UV spectra and generates a localized ion charge center. These species do not fragment due to rearrangement because of this localization in much the same manner, as substituted benzenes and indoles do not fragment, in general, upon ionization. Additionally, Linder *et al.*[16(b)] have reported FTIR spectra of valine, leucine, and isoleucine. Unfortunately, we cannot compare our spectra with theirs because their spectra are taken with molecules at ~525 K. At such a temperature, isomer structures are not present in the gas phase and the CH, NH, and OH features at greater than 3000 cm^{-1} are indicative of free nonhydrogen bonded moieties.

Note also that if these assignments are correct, normal vibrational calculations at any level of harmonic theory would not support these results well. The appearance of positive, negative, or indeed even no IR spectra for given fragment mass channels depends on the details of the potential energy surface for a particular isomer. The surfaces schematically represented in Figs. 3 and 4 are sufficiently general that any of the above spectral outcomes are reasonable.

V. CONCLUSIONS AND SUMMARY

The most fundamental conclusion to be drawn from these studies is that single photon ionization of aliphatic amino acids can be employed to detect isomeric species IR spectroscopy of any (not just aromatic) amino acid in the gas phase. Thus, a UV chromophore is not required to be present to study the vibrational spectroscopy of biologically interesting molecules. The ionization step at 10.5 eV is without fragmentation, but the nascent parent ion undergoes a rearrangement releasing sufficient energy to surmount barriers to fragmentation. Each conformer of a given amino acid can have different fragmentation patterns associated with the intramolecular NH_2 and OH hydrogen bonding patterns for the isomers accessed. Of course, the different isomers possess different IR spectra in the range of 2500–4000 cm^{-1} due to the different CH, NH, and OH modes in the various structures. Mode mixing occurs in the usual CH mode region as OH and NH modes are shifted and broadened into this energy range due to strong intra-molecular N—H and O—H hydrogen bonds. Vibrational energy in the molecules prior to ionization causes fragmentation channels to open and affects the intensity of the mass spectral features in different mass channels. These observations taken together can give at least qualitative structural information for these isolated amino acids.

We are now expanding such studies to include other amino acids, sugars, peptides, saccharides, and glycopeptide systems.

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