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Mid-IR spectroscopy of protonated leucine methyl ester performed with an FTICR or a Paul type ion-trap

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Abstract

The protonated leucine methyl ester structure was probed using mid-infrared multiphoton dissociation (IRMPD) spectroscopy performed at CLIO, the Orsay Free Electron Laser facility. A first experimental spectrum was obtained with a Fourier-transform ion-cyclotron-resonance mass spectrometer with ions generated through a MALDI process. A second spectrum was recorded with ions generated using ElectroSpray Ionisation and trapped in a Paul ion-trap. These two experimental spectra are analyzed and compared with infrared absorption spectra derived from hybrid density functional theory calculations. The two IRMPD spectra are in excellent agreement, although the one recorded with the Paul ion-trap presents a better resolution with an fwhm of the IRMPD bands of 20–25 cm^{-1}. Comparison with the calculated IR absorption spectra clearly shows that only the lowest energy isomer is formed under both experimental conditions. The CO stretch, together with two vibrational modes of the ammonium group were the three infrared signatures identified in the photon energy range explored here.

Keywords: Tandem mass spectrometry; Infrared spectroscopy; Protonated; Amino acid derivatives; Leucine

1. Introduction

The photofragmentation [1] of ions in trapping devices is recognized as a valuable tool for the structural characterisation of ionized species. Both fundamental and analytical aspects are important since the spectroscopic studies from the infrared through the UV strongly rely on the understanding of the kinetics associated to the photo-induced fragmentation. In this context, an important contribution of Chava Lifshitz was to address fundamental questions associated to the photo-induced fragmentation process. Time-resolved photodissociation (TRPD) experiments were carried out on polycyclic aromatic hydrocarbons [2] and small peptide cations [3] with a dedicated experimental setup featuring a Paul trap associated to a reflectron time-of-flight to combine the storage capabilities of the former with the speed and resolution of the later. This work of C. Lifshitz demonstrated that a Paul-trap device is suitable for decay time investigations on the millisecond time scale. Chava Lifshitz's TRPD studies of peptide ion fragmentation provided a clear indication that the internal energy that is acquired upon UV excitation is randomized prior fragmentation.

The mid-infrared is a particularly interesting energy domain for the spectroscopic interrogation of the molecular structure. Providing the attachment of weakly bound rare gas atoms, for example, molecular ion fragmentation may occur upon the absorption of a single photon. Since the pioneering works in the 1980 [4], this ion-tagging approach has been successfully used for deriving IR spectra of a large variety of molecular [5], transition metal complexes, [6] and cluster ions [7]. Recent results on the systematic investigation of the perturbation induced by the weakly bound messenger confirm that the structure of the molecular ion might be affected by the messenger [8], like in

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the case of cationic species trapped in rare gas matrices [9]. One can also directly investigate the structure of bare molecular ions using the optical power provided by infrared free electron lasers (IR-FEL) which is particularly well-suited for inducing an infrared multiphoton dissociation (IRMPD). In this context, FELIX [10] and CLIO [11] free electron laser facilities are particularly interesting since they present an easily and broadly tuneable output in the 100–2500 cm^{-1} energy range thus offering an interesting complement to the 2–5 μm IR-OPO sources. Infrared fingerprint of a large variety of molecular ions has been obtained through IRMPD spectroscopy even in cases where fragmentation requires the absorption of several tens of IR photons. It should be noted that IRMPD can also be performed with infrared table-top lasers in the 3000–4000 cm^{-1} energy range [6,12], provided that the dissociation energy is relatively low. Finally, it should be recalled that the mechanism of the IRMPD process of molecular ions strongly relies on an efficient intramolecular vibrational re-distribution (IVR) of the resonantly absorbed photon energy. Following the resonant IR excitation of the mass-selected ion, an internal energy distribution must take place for allowing the subsequent photon absorption. Considering the evolution of the density of vibrational states with the internal energy, this IVR process is particularly critical after the absorption of the very first photon, especially in the case of small molecular ions. As a matter of fact, whereas the IRMPD spectra of the larger bare polycyclic aromatic hydrocarbons ions have been recorded at FELIX, the tagged-ion approach was used for deriving the IR spectrum of benzene cation [13].

Exploiting modern ion sources [14] and the most advanced tandem mass spectrometric capabilities for the transfer of biological substrates to the gas phase and the subsequent mass-selection, and detection of the ions of interest are important issues. For the first IRMPD spectra [15] of polycyclic aromatic hydrocarbon ions recorded at FELIX, a Paul type [16] quadrupole ion-trap was used, and its content was extracted and analysed with a time-of-flight mass spectrometer. Thermal or laser vaporization of a solid target, and subsequent ionization using an ArF excimer laser focused at the center of the trap allowed for the study of polycyclic aromatic hydrocarbons ions, and a large variety of transition metal complexes [17] or clusters [18] cations were also formed using a pick up of vaporized metal in a pulsed Argon expansion seeded with appropriate ligands. Linear multipole traps have also been coupled to IR-FEL for IRMPD spectroscopic investigations, [19] and this instrument was coupled to a cluster source. More recently, a large variety of ion sources have been used with Fourier-transform ion-cyclotron-resonance mass spectrometers both at FELIX [20] and CLIO [21,22].

We herein present the IRMPD spectra of protonated leucine methyl ester ($LeuMeH^+$) obtained under two experimental conditions. IRMPD of $LeuMeH^+$ ion was first performed with our FTICRMS instrument, and a matrix assisted laser desorption ionisation (MALDI) type source was used to produce the desired ions. The resulting IRMPD spectrum is compared to the one obtained with our modified Bruker Esquire 3000, a Paul ion-trap type instrument, where the ions are produced through Electro-Spray Ionisation. Comparison of these two IRMPD spectra with calculated IR absorption spectra of the lowest energy isomers of $LeuMeH^+$ strongly suggests that only the most stable isomer is formed under the two experimental conditions. Three bands are found to give a clear cut diagnostic: a vibrational mode involving a rocking motion of the ammonium group, a second one associated to the umbrella vibration of the ammonium group, and finally the carbonyl stretching mode. IRMPD bands are found to be broader using our FTICRMS instrument than with our modified Bruker Esquire 3000 Paul ion-trap. This finding suggests that a narrower ion internal energy distribution is achieved through collisions with helium buffer gas in the Paul ion-trap. We also found that under FTICRMS conditions, the optimal focusing is the result of a compromise between the maximization of the overlap with the ion cloud, on one hand, while keeping a sufficiently high fluence for achieving multiple photon absorption on the other. On the other hand, a much higher efficiency was observed using the Paul ion-trap with which large fragmentation yield, up to 100%, can be achieved within a single IR-FEL macropulse. The present results constitute part of an ongoing effort of our group and collaborators for the determination of infrared signature of conformational changes of small amino acids derivatives, and that both the protonated and the proton bound dimers of glycine, alanine, valine and leucine methyl esters [23] have been studied by IRMPD spectroscopy under FTICRMS conditions.

2. Methods

Two experimental platforms, one based on an FTICRMS and the other on a Paul ion-trap, were used to perform the IRMPD spectroscopy using the Infrared Free Electron Laser of CLIO (Centre Laser Infrarouge d'Orsay) [11]. Based on a 10–50 MeV electron accelerator, the photon energy of the CLIO IR-FEL is further tuned by adjusting the undulator gap which is placed in the optical cavity. For instance, in the present work, the electron energy was set to 42 or 45 MeV in order to continuously scan the photon energy in the 950–1850 or 1200–2400 cm^{-1} energy range, respectively. The IR-FEL output consists of 8 μs long macropulses fired at a repetition rate of 25 Hz, and each macropulse contains about 500 micropulses, each a few picoseconds long. For a typical IR average power of 500 mW, the corresponding micropulse and macropulse energies are 40 μJ and 20 mJ, respectively. The mean IR power was about 700–800 mW during the 950–1950 cm^{-1} energy scan, while it was slightly decreased from 800 to 400 mW during the 1200–2400 cm^{-1} energy scan. The laser wavelength profile was monitored while recording the spectra with a monochromator associated to a pyroelectric detector array (spiricon). The IR-FEL spectral width can be adjusted through a tuning of the optical cavity length, and the laser spectral width (fwhm) was less than 0.5% of the central wavelength. For both experimental setups, the same 1 m focal length spherical mirror was used to mildly focus the IR-FEL beam at the center of the ion-trap, and its position was tuned so as maximize the fragmentation efficiency.

The experimental spectrum in the 1200–2400 cm^{-1} energy range was recorded using a transportable FTICRMS, MICRA

(for Mobile ICR Analyser) [24]. The corresponding experimental setup has been described in details previously [25]. The important feature of MICRA is that it is based on a 1.24 T permanent magnet, and that the magnetic field is perpendicular to the bore of the magnet, and this feature has two consequences. First, the IR beam enters perpendicular to the magnetic field, and we will see that this may singularly affect its overlap with the ion cloud. Second, only in-cell or near-cell ionisation can be performed. We have previously described how molecular ions could be generated through a MALDI process in MICRA [26]. A sample was deposited on a metallic holder mounted just outside the ICR cell, 6 mm away from the middle of the nearest trapping plate, and desorption and ionisation were obtained using the third harmonic (355 nm) of a pulsed Nd:YAG laser. Hydrochloride salts of the L-leucine methylester (Aldrich) mixed with α-cyano-4-hydroxycinamic acid (CHCA) as the matrix in a 1:1 mass ratio was compressed into a 1 mm thick pellet, and a piece (∼4 mm × 4 mm) of this pellet was deposited on the holder. Protonated LeuMeH$^+$ ions were mass selected 100 ms after the Nd:YAG pulse, allowed to relax for 100 ms and subsequently irradiated with the IR beam for 1s (i.e., 25 IR-FEL macropulses), and detection was settled 50 ms after the end of irradiation. This duty cycle ending with a quench was repeated eight times for each selected photon energy, and the mass spectrum was the Fourier Transform of the accumulated signal. IRMPD spectra reported in this paper correspond to the fragmentation efficiency $R = -\ln(I_{\text{parent}}/(I_{\text{parent}} + \sum I_{\text{fragment}}))$ as a function of the photon energy.

The experimental spectrum in the 950–1950 cm^{-1} energy range, together with its complement between 800 and 900 cm^{-1}, were recorded using a modified Bruker Esquire 3000 Paul-trap type mass spectrometer. A 10^{-5} M aqueous solution of leucine methyl ester hydrochloride salt was used. ESI conditions used were as follows: a rate of 80 μl/h, a spray voltage of 3500 V, a capillary temperature of 200 °C. A conical hole was made in the ring electrode in order to allow the optical access to the center of the trap. The IR-FEL beam enters through a ZnSe window oriented near to the Brewster angle so as to present the maximum transmission. Multistage mass spectrometry was carried out using the standard Bruker Esquire Control (version 5.2) software. Within the MS1 step, single isotope was mass-selected in a window of 1 Da for the LeuMeH$^+$ ions of interest. The control of the irradiation time of the ions was performed using the MS2 step where the excitation amplitude was set to zero, and the associated output trigger was used to control the optical shutter, which was then opened for a controlled number of the IR-FEL macropulses. Mass-selected LeuMeH$^+$ were irradiated with a single IR-FEL macropulse. Mass spectra were recorded after 10 accumulations, and this sequence was repeated 7 times for each photon energy, which was increased by steps of ∼4–5 cm^{-1}.

Isomer search for LeuMeH$^+$ was performed at the B3LYP/6-31+G(d,p) level of theory using the Gaussian 98 package, [27] and the infrared absorption spectra of the two lowest lying isomers were calculated within the harmonic approximation. Providing the use of an appropriate scaling, hybrid DFT methods such as B3LYP have been shown to outperform other DFT methods as well as traditional ab initio approaches to describe both positions [28] and relative intensities [29] of IR bands. As far as the positions are concerned, it was shown that a dual scaling approach [28] significantly improves the comparison with experiment. Indeed, high energy modes, typically above 1800 cm^{-1}, essentially involve localized hydrogen stretches which can be expected to be more anharmonic than the vibrational modes below 1800 cm^{-1}. As a result, providing the use of a polarized-valence triple zeta basis set, the scaling factor associated to hybrid density functionals such as B3LYP are typically 0.98–0.99 below 1800 cm^{-1} and 0.96–0.97 above 1800 cm^{-1}. A scaling factor of 0.98 was applied to all calculated frequencies reported in the present article. It should be noticed that the same value was also determined using a regression analysis for comparing our calculated frequencies with gas phase experimental values in the same wavelength range [21,26].

3. Results and discussion

The IRMPD spectra of LeuMeH$^+$ recorded using our FTICR and Paul ion-trap are presented in Fig. 1a and b, respectively. One can notice that there is a good agreement between the two sets of data, although the IRMPD spectrum recorded with our

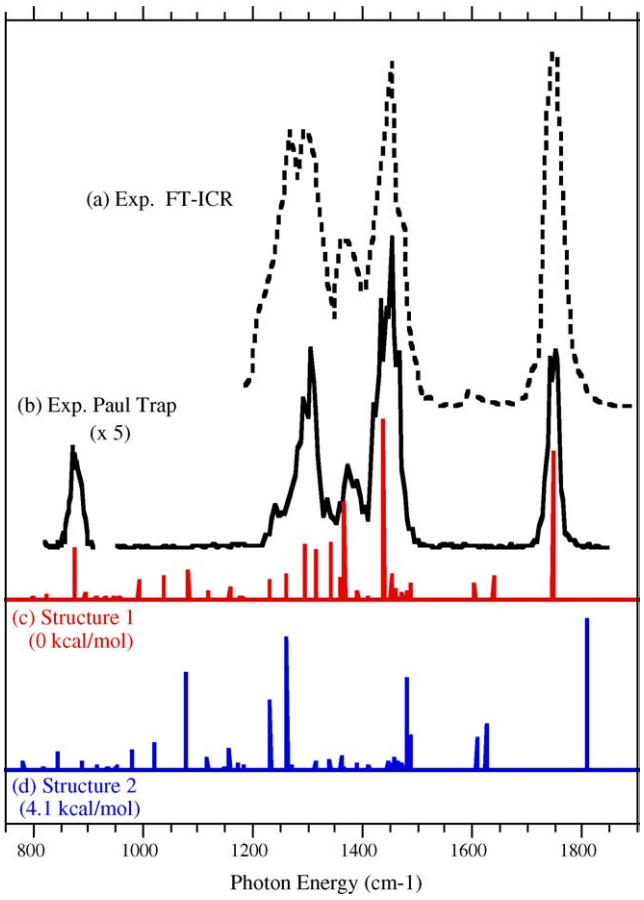

Fig. 1. IR spectrum of protonated leucine methyl ester LeuMeH$^+$. Experimental IRMPD spectrum recorded using 25 IR-FEL macropulses with our FTICR (a) and only one IR-FEL macropulse with our Paul ion-trap (b) compared to the DFT calculated IR absorption spectrum of the lowest energy conformer **1** (c) and a higher energy conformer **2** (d).

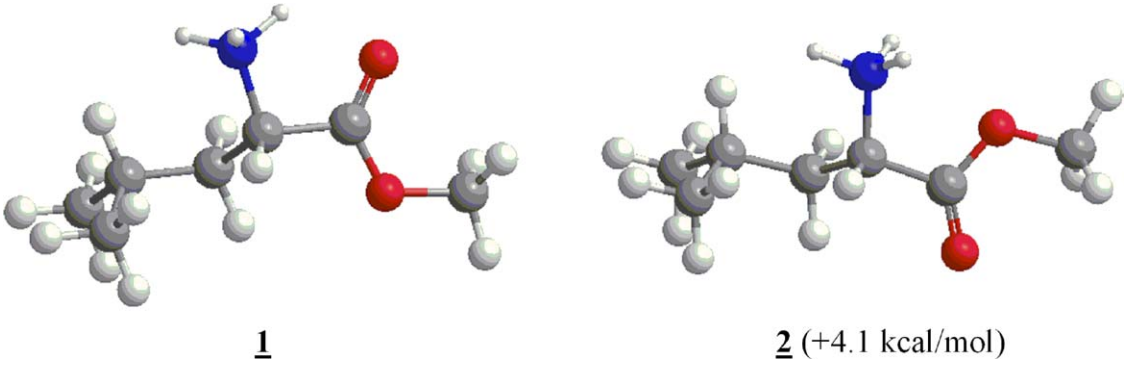

Fig. 2. Optimized structures of the two lowest energy conformer of protonated leucine methyl ester LeuMeH$^+$.

Paul ion-trap (Fig. 1b) offers a better resolution. Thus, in the following, we will only mention the position of the IRMPD bands obtained with the Paul ion-trap. The IR absorption spectra of the two lowest energy structures of LeuMeH$^+$ are given in Fig. 1c and d. For both structures **1** and **2**, given in Fig. 2, protonation occurs on the most basic site amino group which is either coordinated to the oxygen of the carbonyl group (**1**) or to the one of the methoxy group of the ester function (**2**). In agreement with the extensive structural studies of protonated amino acids derivatives, the structure corresponding to the ammonium group interacting with the carbonyl **1** was found to be the most stable. In the present case, the structure **2** corresponding to the solvation of the ammonium by the methoxy group was found 4.1 kcal/mol higher in energy.

The comparison of IRMPD and calculated IR absorption spectra, and the subsequent conformer assignment can be a delicate task in some cases for at least two reasons. First, one could anticipate that due to the multiphotonic absorption process, the resulting IRMPD bands might appear red-shifted with respect to the corresponding one photon IR absorption band. Nevertheless, it appears that when the dissociation threshold is relatively low like in the present case, this red-shift effect is small. Second, calculated fundamental vibrational frequencies must be scaled in order to correct for the harmonic approximation. In our previous work on transition metal complexes [21–23], we were able to derive a scaling factor by comparing the calculated frequencies of the ligands to the available corresponding gas phase absorption spectra. Considering the wavelength range (800–1600 cm^{-1}) for these metal complexes, the probed vibrations were the ones of the free ligands slightly shifted because of the interaction with the metal cation. Unfortunately, no gas phase infrared absorption spectrum was available for leucine methyl ester. Nevertheless, a conclusive conformer assignment can be made, which in turn confirms the choice of the 0.98 scaling factor.

The two IRMPD spectra (Fig. 1a and b) present three bands in the 1200–1500 cm^{-1} energy range, and one additional band corresponding to the carbonyl group observed at 1748 cm^{-1}. Furthermore, an additional IRMPD band was identified using our Paul-trap mass spectrometer at 877 cm^{-1}. As can be seen in Fig. 1, no scaling factor for conformer **2** would be successful at simultaneously matching all the major features of the spectrum, whereas the matching for conformer **1** using a scaling factor of 0.98 is excellent. In particular, one can see that the scaled IR absorption spectrum presents bands in excellent agreement with the one observed at 877 and 1748 cm^{-1}. This later IRMPD feature is interesting for the structural characterization. Indeed, as can be seen in Fig. 1, the two low-energy isomers can be clearly differentiated based on their calculated IR spectra, especially in the region of the carbonyl stretch. In the lowest energy conformation **1**, the carbonyl stretching mode (1746 cm^{-1}) is significantly red-shifted as compared to its position (1809 cm^{-1}) in the other isomer (Fig. 1d) where the carbonyl is free whereas the ammonium group is solvated by the oxygen of the methoxy group. The region below 1200 cm^{-1} is also interesting for the conformer assignment. Indeed, no IRMPD signal was observed between 950 and 1200 cm^{-1} using the Paul-Trap, whereas conformer **2** presents a strong absorption feature at 1080 cm^{-1}. The calculated intensity of this band (140 km/mol) is such that it should lead to a significant IRMPD signal. We can thus safely assign the observed IRMPD spectra to conformer **1**.

This conformer assignment is also supported by the thermochemical calculations predicting that conformer **1** should be strongly favored. Thus, it seems that only the lowest energy isomer is formed either through the MALDI process under FTICRMS conditions or through ESI as observed in the Paul ion-trap. It should be noted that in our previous IRMPD spectroscopic investigations of molecular ions either generated through MALDI [26] or electron impact, only the IR signature of the lowest energy isomer was systematically observed. The only exception to date was observed in the case of protonated cytosine, which presents two competitive tautomers separated by only ∼1 kJ/mol. All the features of the IRMPD spectrum of protonated cytosine [30] generated by ESI can be assigned with the calculated IR absorption spectrum of the lowest energy tautomer, except one (corresponding to a free CO stretch) which is clearly the IR signature of the simultaneous presence of the other low-lying tautomer at only ∼1 kJ/mol.

The positions of the maxima associated to the two IRMPD spectra are reported in Table 1, in which the positions of the calculated IR active vibrational modes of the two conformers are also given. As said above, the strong IRMPD yield observed at 1748 cm^{-1} (Table 1) can be assigned to the CO stretching mode of the lowest energy conformer **1** calculated at 1746 cm^{-1}. No

Table 1
IR bands of protonated leucine methyl ester LeuMeH$^+$

Vib. modes	Experiment		DFT calculation	
	Paul-trap	FTICR	Conformer **1**	Conformer **2**
–NH_3^+ rock	877		875 (0.28)	1080 (0.64)
C–H bend	1289	1268	1230 (0.1)	1232 (0.45)
	1305	1291	1295 (0.30)	
	1372	1361	1367 (0.54)	
–NH_3^+ umbrella	1452	1453	1438 (1)	1481 (0.61)
–NH_3^+ as-deform		1592	1603 (0.08)	1608 (0.21)
			1639 (0.13)	1625 (0.30)
C=O stretch	1748	1743	1746 (0.83)	1809 (1.00)

The positions (cm^{-1}) of the IRMPD bands observed using our Paul and our FTICR ion-trap are given in columns 2 and 3, respectively. The positions and relative (intensities) of the most IR active bands as calculated for the two confirmers 1 and 2 are given in columns 4 and 5, respectively.

IRMPD signal was observed for higher photon energies suggesting that the second conformer **2** is not populated in our experimental conditions.

The spectral assignment is more difficult for the IRMPD bands observed in the 1200–1500 cm^{-1} energy range, although one can notice that within the two experimental conditions, the strongest IRMPD signal (1452 cm^{-1}) was observed when the IR-FEL was in resonance with the most strongly IR active mode of isomer **1**, calculated at 1438 cm^{-1}. Thus, this IRMPD band can be assigned to the NH_3 umbrella mode of the ammonium group, although contributions of moderately IR active modes calculated at 1452 and 1488 cm^{-1} cannot be excluded. As can be seen in Table 1, the NH_3 umbrella mode of the ammonium group is also a good conformational diagnostic since it is significantly more red-shifted by the coordination to the carbonyl (1438 cm^{-1} in **1**) than to the methoxy group (1481 cm^{-1} in **2**). An additional IRMPD band with a maximum observed at 1305 cm^{-1} using the Paul ion-trap is also observed under FTICR conditions, but it is significantly broader and it is found slightly red-shifted in the later case (1291 cm^{-1}). The same observations can be made for the IRMPD band observed at 1372 cm^{-1} with the Paul ion-trap, which is broader and appeared red-shifted (1361 cm^{-1}) in FTICR conditions. An inspection of Table 1 clearly shows that LeuMeH$^+$ presents a large number of IR active modes in this energy range, essentially corresponding to CH bends. A clear assignment of these two IRMPD bands is therefore difficult since they might be the results of absorptions through several IR active modes.

Finally, the 800–900 cm^{-1} energy range (see Fig. 1b) has been explored using the Paul ion-trap. An IRMPD band observed at 877 cm^{-1} seems to further confirm the presence of isomer **1**, since the corresponding calculated IR absorption spectrum presents an IR active band at 875 cm^{-1}. The associated normal mode is a combination of several local modes with a strong component on a rocking type deformation of the ammonium group. Comparison of the two calculated spectra of structures **1** and **2** shows that this ammonium rocking mode is also a good infrared diagnostic for the structural characterization of LeuMeH$^+$.

A unique fragmentation channel was observed when the IR-FEL was in resonance with an IR active mode of the LeuMeH$^+$ ion. It corresponds to the formation of (*i*-butyl)CH=NH_2^+ immonium ion (m/z = 86) ions and to the loss of [2C,2O,4H]. Immonium ion has also been shown to be the major fragment ion when amino acid derivatives are subjected to collision induced dissociation [31], although acylium b-ions were also observed under low energy CID in the case of small peptides [32]. Theoretical study of the fragmentation pathways of protonated glycine [33] suggests that fragmentation may occur sequentially, a loss of water leading to acylium which spontaneously looses CO leading to immonium. Acylium fragments were not observed in the present case where fragmentation of LeuMeH$^+$ ions was induced by multiple infrared photon absorption under FTICRMS or Paul-trap conditions.

The fragmentation occurring when a molecular ion is excited through an infrared multiple photon process is generally viewed a result of successive cycles involving infrared absorption through a resonant vibrational mode, followed by an intramolecular vibrational re-distribution of the energy. Although there might be a debate whether or not a second absorption regime takes place at high internal energy, relying on the presence of a quasi-continuum with a high density of vibrational energy states, it is generally recognized that infrared multiple photon absorption is a slow heating process, and several IRMPD spectroscopic investigations already provided evidences that statistical distribution of the internal energy occurs prior dissociation. Nevertheless, the rate of the multiple absorption process might depend on the experimental conditions and more precisely on the overlap of the ion cloud with the infrared laser. It is generally believed that ion confinement in a Paul ion-trap device is further enhanced by collisions with the helium buffer gas. As a result, the overlap of the IR-FEL with the ion cloud in our Paul ion-trap device is likely to be efficient. This is supported by the fact that a 100% fragmentation yield can be observed after irradiation of molecular ions with a single macropulse of the IR-FEL. As a matter of fact, in the present case (see Fig. 1), the fragmentation yield is approximately five times greater using the Paul ion-trap device than under our FTICR conditions as can be seen in Fig. 1. In the later case, the overlap between the IR-FEL and the ion cloud is the result of a compromise. While maintaining a high fluence through the focalization of the IR-FEL, one has to keep in mind that the motion of the ion in the ICR cell is a combination of the cyclotron motion with two large amplitude motions [34]. First, axial "trapping" oscillation occurs along the magnetic axis, and second rotational "magnetron" motion occur about the magnetic axis. An order of magnitude of the amplitude of these two oscillations can be given by monitoring the fragmentation yield of an ion while translating the position of the waist of the laser beam from its optimal position. The corresponding results are given in Fig. 3 for $Fe(CO)_5^+$ when the laser was in resonance with the CO stretch. At this photon energy, the laser beam waist diameter has been measured to be 0.7 mm. The fragmentation yield of $Fe(CO)_5^+$ while translating the position of the waist of the laser beam perpendicular to the magnetic axis (Fig. 3b) gives an order of magnitude of the amplitude (3–4 mm) of the "magnetron" motion of the ion about the magnetic axis. Considering that

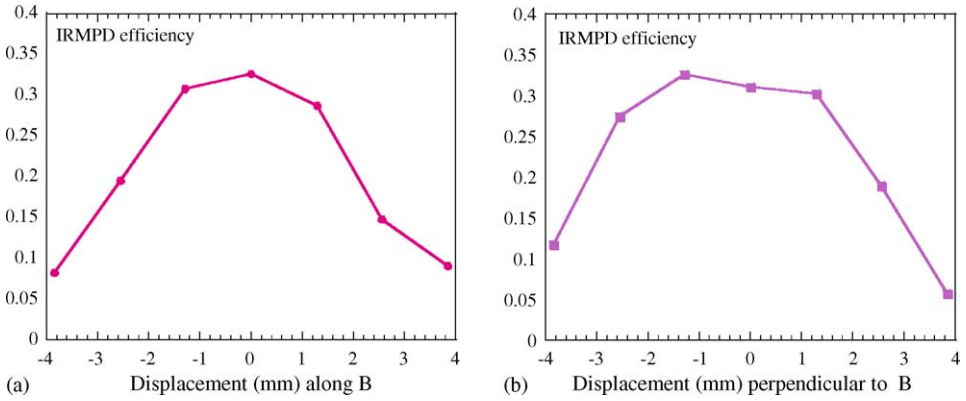

Fig. 3. IRMPD efficiency as a function of the displacement of the laser waist in the ICR cell along the magnetic axis (a) or perpendicular to it (b). $Fe(CO)_5^+$ ions were mass-selected and irradiated with the IR-FEL in resonance with the CO stretch, the laser beam waist at this photon energy was approximately 0.7 mm.

this magnetron motion is slow (ms) as compared to the length (8 μs) of the IR-FEL macropulses, one can understand that only a small percentage of the ion cloud overlaps with a single IR-FEL macropulse in our FTICRMS conditions. The order of magnitude of the axial "trapping" motion along the magnetic axis is also about 3–4 mm as can be seen in Fig. 3a where the fragmentation yield of the $Fe(CO)_5^+$ ion is plotted against the displacement of the beam waist along the magnetic field. The consequence of the "trapping" motion on the ion-cloud/laser overlap is also important since its period (tens of micro second for *m/z* of the order of 100) is of the order of magnitude of the length of the train of picosecond pulses of the IR-FEL. As a result, the overlap of a given ion with the train of picosecond pulses might be singularly reduced which would suggest that the infrared multiple photon absorption process is slower under FTICR than Paul ion-trap conditions. This could explain that while different fragmentation channels [35] can be observed upon the IR-FEL irradiation when molecular ions are trapped in a Paul device, only the lowest energy fragmentation channel is generally observed under our FTICR conditions. When several fragments were observed under these conditions, like in the case of $Fe(butene)^+$ [36], where both $Fe(butadiene)^+$ and Fe^+ were observed, we showed that the later was formed through a sequential IRMPD process: Fe^+ ions were only observed when the IR-FEL was tuned at a frequency corresponding to an IR active mode of both $Fe(butene)^+$ and $Fe(butadiene)^+$. Multiple fragmentations were observed under our FTICR conditions when two fragmentations closely compete like in the case of proton-bound dimers [37] and in the case of $Fe((CH_3)_2O)_2^+$ where the direct loss of methyl, which is a direct but endothermic process, was in competition with a stepwise rearrangement leading to the exothermic loss of methane [22]. It was interesting to observe that the branching ratio of the two fragmentation channels (i.e., $-CH_3/-CH_4$) did not depend upon which IR active mode of $Fe((CH_3)_2O)_2^+$ was in resonance with the IR-FEL, which seemed to confirm that statistical distribution of the internal energy occurs prior fragmentation.

The resolution of the IRMPD spectrum also strongly depends on the experimental conditions. The IRMPD bandwidth observed under FTICRMS conditions (fwhm = 40 cm^{-1}) for $LeuMeH^+$ are of the same order of magnitude as what has been observed previously with the same experimental setup [36]. Considering that the IR-FEL bandwidth is about 0.5% of the central wavelength, and based on the comparison with IR absorption spectra of neutral species at room temperature, it was suggested that the IRMPD bandwidth might be essentially controlled by the rotational contour of the vibrational band [36]. When IRMPD spectroscopy is performed using our Paul ion-trap, the IRMPD bands are significantly narrower (fwhm = 25 cm^{-1}), which is likely to be the result of the efficient collisional thermalization with the helium buffer gas. Only radiative cooling occurs under our FTICR conditions. From the above discussion on overlap between the ion-cloud and the IR-FEL, one would anticipate that the internal energy distribution within the ion cloud might be further broadened through the sequential slow heating process from the successive macropulses. Furthermore, it should be noted that in the present case, $LeuMeH^+$ were allowed to relax for only 100 ms, since at the second timescale a significant decrease of the corresponding signal was observed with a concomitant formation of proton bound dimer of leucine methyl ester, probably resulting from collisions between $LeuMeH^+$ and neutral LeuMe produced in excess during the MALDI process.

4. Summary

IRMPD spectrum of protonated leucine methyl ester has been recorded in the mid-IR range (800–2400 cm^{-1}). The infrared band associated to the CO stretch, together with two associated with deformation of the ammonium group provide the infrared signature of the conformation. Comparison of these experimental data with computed IR absorption spectra of the two lowest lying conformers clearly shows that only the lowest lying isomer, characterized by the ammonium group coordinated to the carbonyl group, is formed under both MALDI and ESI conditions. This IRMPD spectroscopic investigation of protonated leucine methyl ester under two experimental conditions allowed for a discussion of the pros and cons of our two experimental setups. In particular, the highest overlap between the IR-FEL beam and the ion cloud, together with the highest IR-FEL fluence, which can be achieved with a Paul trap, might be encouraging in the perspective of the extension of the IRMPD spectroscopy to the NH and OH stretch region with tabletop lasers.

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