Chemical Physics Letters 502 (2011) 29–36

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

Characterization by mass spectrometry and IRMPD spectroscopy of the sulfoxide group in oxidized methionine and related compounds

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ARTICLE INFO

Article history:
Received 26 October 2010
In final form 2 December 2010
Available online 5 December 2010

ABSTRACT

Methionine protein residues are prone to oxidation. To unravel the controversy about the mechanism of its one-electron oxidation, we characterised the main biological product, methionine sulfoxide, using mass spectrometry and IR multiple photon dissociation spectroscopy.

Gas phase IR spectra in the 800–2000 cm^{-1} range of protonated methionine and its sulfoxide were recorded and compared to those computed for the lowest energy structures. The signature of the S=O bond was clearly identified at around 1000 cm^{-1}. Oxidation of methionine–lysine dipeptide by ·OH radicals in the presence of catalase revealed the formation of methionine sulfoxide upon one-electron oxidation.

1. Introduction

The development of all diseases involves the production of oxygen and nitrogen free radical species that play a relevant role in the defence against infectious agents [1]. However, oxidative stress leads to the accumulation of oxidized proteins, hence to troubles in tissue homeostasis (induction of cell death or of cell proliferation, necrosis, etc.). One of the most frequent modifications in oxidized proteins is that of the residue methionine **(Met)**, an essential amino acid, into its sulfoxide form **(MetSO)** [2]. Its oxidation is a highly damaging event associated to the aging process and to structural modifications in neurodegenerative diseases (neuritic plates in Alzheimer's disease [3,4], destabilization of the Prion protein [5]). **MetSO** can be 'repaired' by methionine sulfoxide reductases [6] thus its formation could be protective by acting as a molecular switch to detoxify oxidants and protect important regions of the protein.

Strangely enough, the transients formed in the oxidation of Met by ·OH radicals have been characterized in peptides and proteins [7–9], whereas large doubts remain as for the final oxidized forms. Recently the one-electron oxidation of methionine amino acid in solution has been re-investigated [10]. The authors concluded that methionine sulfoxide was not created by the reactions of ·OH radicals but only by two-electron processes (H_2O_2, oxygen). However in studies of anaerobic one-electron oxidation of peptides or proteins, the only final compound coming from the methionine residue is **MetSO** [11–14].

The aim of the present study is to identify and to characterise by a combination of tandem mass spectrometry (MS/MS) coupled to Infra-Red Multiple Photon Dissociation (IRMPD) spectroscopy the sulfoxide function and to investigate the oxidation of a dipeptide, methionine–lysine, before moving on to similar experiments with larger Met-containing peptides. We first used commercially available **Met** and **MetSO**. Moreover, the relatively small size of the molecules allowed the use of DFT calculations to reproduce the IR spectra with rather large bases.

These experiments have been performed with a high power and widely tunable IR source, i.e. a free-electrons laser (FEL) at the CLIO (Centre Laser Infrarouge d'Orsay) facility in Orsay (France) [15]. A rapid increase of the internal energy is induced through an efficient resonant multiple photon absorption process [16–18].

2. Materials and methods

2.1. Reagents and solutions

Methionine, methionine sulfoxide and Catalase (**Cat**) (bovine liver, suspension in water) were obtained from Sigma (France) and used without purification. The catalytic activity of **Cat** was checked using a solution 1 mM of H_2O_2. One microliter of the suspension was added to the solutions before irradiation.

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doi:10.1016/j.cplett.2010.12.012

2.2. MS operations

The mass spectra were recorded using a modified Paul ion trap (Bruker, Esquire 3000+). 100 μM 50:50 water:methanol solutions were employed for recording the mass spectra.

Ions were generated by electrospray ionisation (ESI) under the following conditions: a flow rate of 150 μL/h, a dry gas flow of 8 L/min, a nebulizer pressure of 1.5 bar, a spray voltage of −4500 V, and a drying gas temperature of 150 °C. Multistage mass spectroscopy was carried out using the standard Bruker Esquire Control software. CID-MS^2 fragmentation mass spectra were produced by collision with He gas inside the Paul trap using a radiofrequency amplitude of 0.5 (**[(Met)H^+]** and **[(MetSO)H^+]**) or 0.6 (**[(Met–Lys)$H]^+$**) and derivatives]. IRMPD spectra of protonated methionine **[(Met)H^+]**, protonated methionine sulfoxide **[(MetSO)H^+]**, protonated methionine–lysine in the non-oxidized and oxidized forms ((**[(Met–Lys)$H]^+$**) and **[(MetSO–Lys)$H]^+$**) were obtained through MS^2 experiments where precursor ions were mass selected in a 5 Da mass window, the excitation RF amplitude was set to zero and the ions were then irradiated for 1 s.

2.3. IRMPD spectroscopy

IR multiple photon dissociation (IRMPD) is a multi-step resonant absorption process relying on intramolecular vibrational energy redistribution (IVR) [19]. Infrared spectra are obtained by monitoring the abundance of parent and fragment ions. If F is the sum of the abundances of the fragment ions produced by IRMPD and P the one of the parent ion, our IRMPD spectra correspond to the plot of $-\ln[P/(F+P)]$ as a function of the IR wavenumber.

The IRMPD spectra in the 800–2000 cm^{-1} energy range have been obtained with the IR beam of the CLIO FEL coupled to the modified Paul ion trap. Details on the performance of our modified Bruker Esquire 3000+ were already published [20]. Different electron energies were used (42–45 MeV). Typical average powers were about 1 W around 1000 cm^{-1} and 0.4 W near 2000 cm^{-1}.

2.4. γ irradiation

γ-irradiations were carried out using the panoramic ^{60}Co γ-source IL60PL Cis-Bio International (France) in the University Paris-Sud 11 (Orsay, France). The dose rate was determined by Fricke dosimetry [21] and kept constant at 30 Gy min^{-1}. Samples were gently purged while stirring under a N_2O atmosphere for approximately 60 min before irradiation. Nitrous oxide was delivered by ALPHA GAZ. Its global purity is 99.998%. Water was purified using a Millipore or Elga maxima system (resistivity 18.2 MΩ cm).

All irradiations were performed at room temperature.

The well-known method of scavengers [21] allows a quantitative production of free radicals by stationary γ-radiolysis according to the following reactions. The chosen oxidant species was the ·OH radical produced by γ radiolysis of N_2O-saturated aqueous solutions:

$$H_2O \rightarrow {}^{\cdot}OH,\ H^{\cdot},\ e^-_{aq},\ H_2,\ H_2O_2,\ H^+,\ OH^- \quad (1)$$

$$e^-_{aq} + N_2O + H_2O \rightarrow {}^{\cdot}OH + OH^- + N_2 \quad (2)$$

The radiation chemical yield (G) is equal to 0.55 μmol J^{-1} (Eqs. (1) and (2)). H atoms are also created in much lower yield (0.05 μmol J^{-1}), which lead to desulfuration of methionine [22]. The H_2O_2 yield is also lower (0.07 μmol J^{-1}). Catalase was added to remove it to prevent two-electron oxidation of methionine [23].

2.5. Computational methods

Potential energy surfaces of protonated methionine [(Met)H^+] and methionine sulfoxide [(MetSO)H^+] were explored using a B3LYP 6-311+G** basis set, because the presence of a sulphur atom requires relatively large basis sets.

The IR linear absorption spectra were computed at the same level of the theory in agreement with other works [24]. The starting geometries were chosen so that various sets of interactions (hydrogen bondings) could be realized. In addition different protonation sites were assayed. Structures resulting from the protonation on the amino group are found to be the lowest in energy. Protonation of **[(Met)H^+]** and **[(MetSO)H^+]** on the sulphur atom and on the sulfoxide group, respectively is significantly higher in energy.

Spectral assignment was achieved by comparing experimental spectra to the calculated IR ones. Calculated bands were convoluted assuming a Lorentzian profile with a 30 cm^{-1} full width at half maximum (FWHM).

All calculations were performed with the Gaussian03 package [25]. In the spectral range of interest (800–2000 cm^{-1}), no scaling factor has been applied to the calculated vibrational frequencies [24].

3. Results and discussion

3.1. Mass spectra of methionine and methionine sulfoxide

The CID-MS^2 spectra of protonated methionine **[(Met)H^+]** (m/z 150) and protonated methionine sulfoxide **[(MetSO)H^+]** (m/z 166) have been recorded (Supporting information Figure S1). Two peaks at m/z 133 and 104 respectively are observed in the CID-MS^2 fragmentation spectrum of **[(Met)H^+]**. The peak at m/z 133 corresponds to the loss of ammonia, which is in competition with the formation of the immonium ion for several amino acids bearing a functional group on the side chain. The peak at m/z 104 corresponds to a common fragmentation to all protonated amino acids, which is the well known sequential loss of H_2O and CO, leading to the formation of the immonium ion [26–28].

Three peaks at m/z 149, 131 and 75, respectively are instead observed in the CID-MS^2 fragmentation spectrum of **[(MetSO)H^+]**. The peak at m/z 149 corresponds to the loss of ammonia, while the less intense one at m/z 131 corresponds to the loss of ammonia plus a water molecule. Finally the peak observed at m/z 75 may correspond to the loss of a heteroatom-containing small molecule from the side chain, which is consistent with one of the three possible decomposition pathways of protonated amino acids [29].

3.2. Experimental IRMPD spectra of methionine and methionine sulfoxide and DFT calculations

The structures of **[MetH$^+$]** and **[MetH$^+$SO]** in the gas phase were investigated by IRMPD spectroscopy and DFT calculations to provide a set of frequencies, which constitutes a useful guide for the assignment of the sulfoxide bands in the irradiated dipeptides.

The experimental IRMPD spectra of **[(Met)H^+]** and **[(MetSO)H^+]** in the 800–2000 cm^{-1} energy range are reported in Figure 1. The IRMPD spectrum of **[(Met)H^+]** is very simple. It is composed of three bands at 1170, 1477 and 1800 cm^{-1}. As expected, that of **[(MetSO)H^+]** is more complex. In addition to the preceding main bands, one can observe a new band at 947 cm^{-1}, 3 bands in the 1100–1400 cm^{-1} region and 4 in the 1400–1500 cm^{-1} one.

To reproduce the IRMPD experimental spectra and to tentatively assign the bands, stable geometries of **[(Met)H^+]** and **[(MetSO)H^+]** were searched at the B3LYP 6-311+G** (5d, 7f) level of theory. Calculated IR absorption spectra of the lowest energy

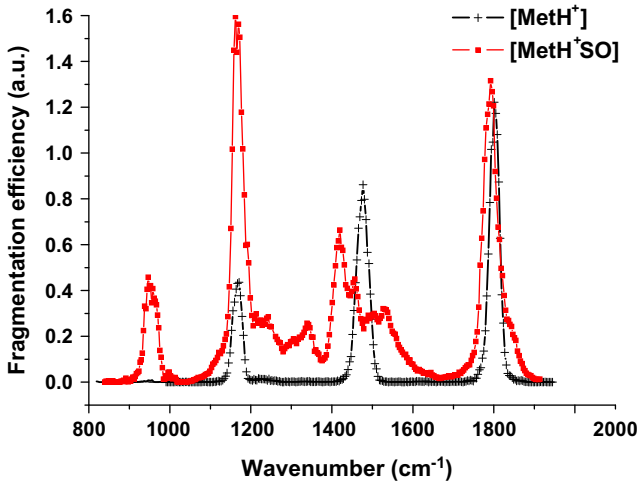

Figure 1. Comparison between the experimental IRMPD spectra of **[MetH$^+$]** and **[MetH$^+$SO]** in the 800–2000 cm^{-1} energy range.

structures of **[(Met)H$^+$]** and **[(MetSO)H$^+$]** along with their optimized structures and their relative energies are reported in Figures 2 and 3, respectively.

Five representative structures of different types of isomers of **[(Met)H$^+$]** are given in Figure 2. Structures **MetH$^+$_1–4** result from the protonation of the amino group, which is the most basic site considering its proton affinity, while isomer **MetH$^+$_5** results from the protonation of the S atom. Rotamers **MetH$^+$_1–2** have the lowest energy in the **[(Met)H$^+$]** Potential Energy Surface (PES). They are stabilized by two strong intramolecular hydrogen bonds, which involve the H-atoms of the protonated amino group, while the acceptor sites are the oxygen atom of the carbonyl group and the sulphur atom [30]. The intramolecular H-bond distances are NH···OC = 2.11 Å and NH···S = 2.13 Å for **MetH$^+$_1** and NH···OC = 2.05 and NH···S = 2.13 Å for **MetH$^+$_2**, respectively. Their calculated absorption IR spectra (Figure 2) are very similar in the 800–2000 cm^{-1} energy region and it is not obvious to assign a precise geometry to our experimental IRMPD spectrum. **MetH$^+$_2** is only 2.52 kJ/mol less stable in energy than **MetH$^+$_1**, and assuming a Maxwell–Boltzman population at room temperature, we may infer that both structures **MetH$^+$_1** and **2** should be populated and both contribute to our experimental IRMPD spectrum.

Isomer **MetH$^+$_3**, which is 32.42 kJ/mol higher than **MetH$^+$_1**, is also characterised by two strong hydrogen bonds between the H-atoms of the protonated amino group and the oxygen atom of the –OH group from one side, and the sulphur atom on the other

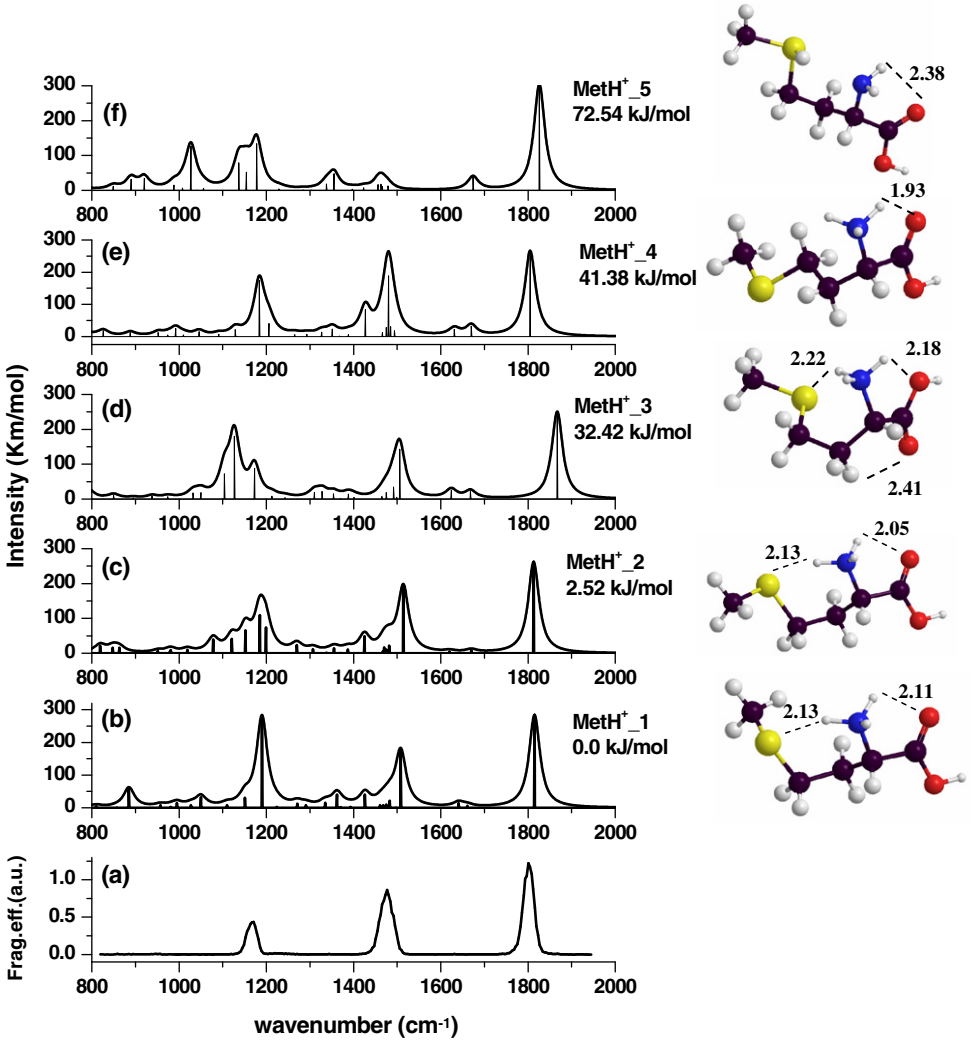

Figure 2. Infrared spectra of **[MetH$^+$]**: (a) Experimental IRMPD spectrum, and (b–f) convoluted calculated IR absorption spectra of representative isomers. Frequency obtained at the B3LYP 6-311+G** level of the theory are unscaled. Representative calculated structures of **[MetH$^+$]** obtained at the B3LYP 6-311+G** level of the theory are reported on the right together with their relative energies. The H-bond distances in the calculated structures are reported in Å.

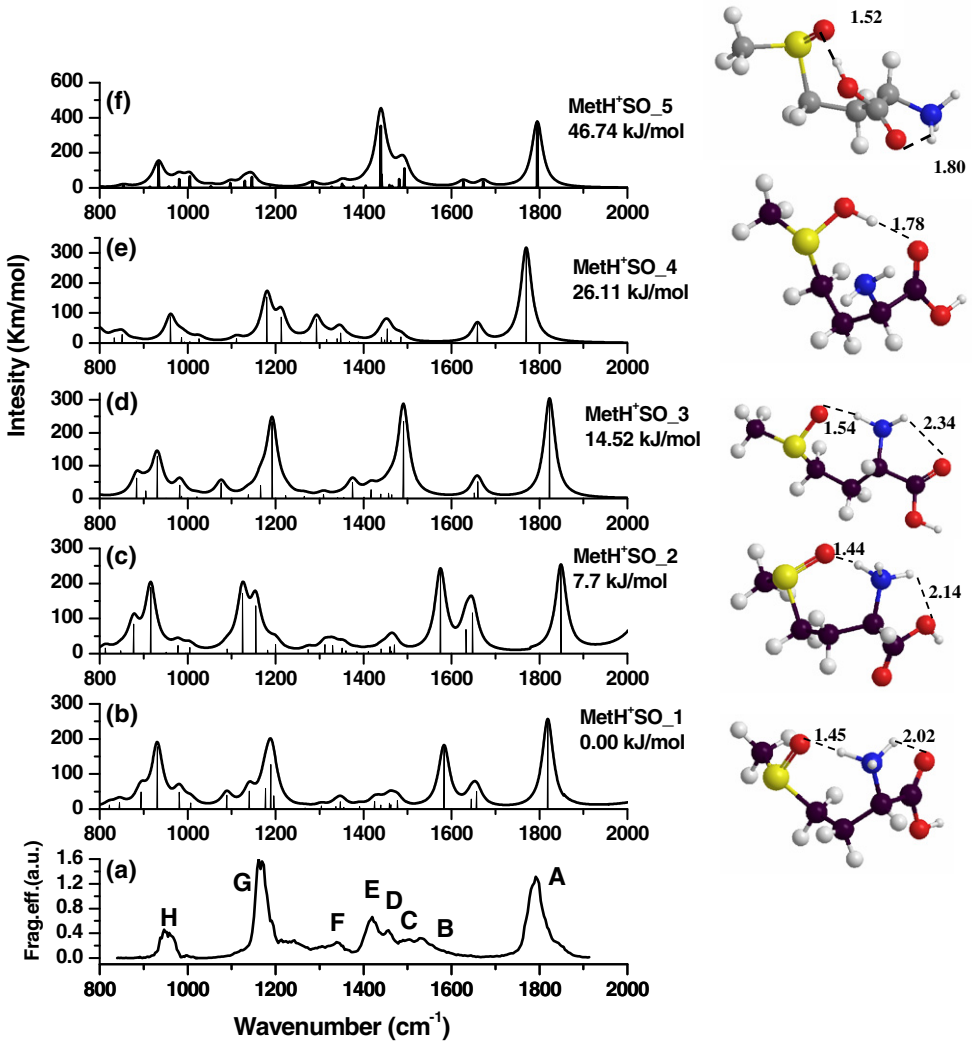

Figure 3. Infrared spectra of **[MetH$^+$SO]**: (a) Experimental IRMPD spectrum, and (b–f) convoluted calculated IR absorption spectra of representative isomers. Frequency obtained at the B3LYP 6-311+G** level of the theory are unscaled. Representative calculated structures of **[MetH$^+$SO]** obtained at the B3LYP 6-311+G** level of the theory are reported on the right together with their relative energies. The H-bond distances in the calculated structures are reported in Å.

site. The H-bond distances are in this case of NH···OH = 2.18 and NH···S = 2.22 Å, respectively.

Isomers **MetH$^+$_4–5**, which are 41.38 and 72.54 kJ/mol, respectively higher than **MetH$^+$_1**, are instead characterised by having just one H-bond. In **MetH$^+$_4** one of the H-atoms of the protonated amino group is involved in a hydrogen bond with the oxygen of the carbonyl group (Figure 2, NH···O = 1.93 Å), and in **MetH$^+$_5** the H atom of the amino group acts as donor towards the O atom of the carbonyl group (Figure 2, NH···O = 2.38 Å).

A tentative assignment of the main IRMPD features of **[(Met)H$^+$]** is proposed in Table 1 by comparing the experimental vibrational frequencies with the ones calculated from the IR active vibrational modes of the lowest energy isomer **MetH$^+$_1**. The ratio between the experimental and the theoretical frequencies are also reported in Table 1.

The band at 1800 cm^{-1} in the experimental IRMPD spectrum can be assigned to the C=O stretching mode. Its position nicely matches with the calculated band in structures **MetH$^+$_1–2** and **MetH$^+$_4**, in which the oxygen atom of the carbonyl group is involved in a hydrogen bond with the H-atom of the protonated amino group. This C=O stretching mode is blue shifted in structures **MetH$^+$_3** (1867 cm^{-1}) and **MetH$^+$_5** (1827 cm^{-1}). This is due to the fact that structure **MetH$^+$_3** does not present this hydrogen bond interaction and structure **MetH$^+$_5** shows a longer NH···O distance (2.38 Å).

Bands at 1662 and 1641 cm^{-1} in the computed spectrum can be attributed to the scissoring vibrational modes of the protonated amino group. They are weakly IR active in the case of **[(Met)H$^+$]** and were not observed in the experimental IRMPD spectrum.

Table 1
Experimental and calculated vibrational frequencies for [MetH$^+$] lowest energy isomer, determined at the B3LYP 6-311+G** (5d, 7f) level of theory, and band assignments. The reported intensities given in parentheses are in km/mol.

Experiment (cm^{-1})	Calculation	Vibrational mode	Ratio (exp/theory)
–	885 (60)	νCC	–
1170	1190 (279)	δCOH, νC–OH, δNH_3^+	0.98
–	1362 (40), 1426 (40)	δCH, νC–OH, δCOH	–
1477	1504 (173)	$\delta_{umb} NH_3^+$	0.98
–	1641 (14)	$\delta_{scissor} NH_3^+$	–
–	1662 (6)	$\delta_{umb} NH_3^+$	–
1800	1815 (285)	νC=O	0.99

The band at 1477 cm^{-1} in the experimental IRMPD spectrum can be attributed to the NH_3^+ umbrella mode of the ammonium group. This band is a good conformational diagnostic of the protonation on the amino group, which is involved in two strong hydrogen bonds. The position of this band is blue-shifted compared to other systems, like the methyl ester of leucine [20], (1452 cm^{-1}) where the protonated amino group is involved in only one strong hydrogen bond with the oxygen atom of the carboxylic group.

Finally the band at 1170 cm^{-1} can be assigned to the bending mode of the C–O–H group. The position of this band matches the ones calculated for structures **MetH$^+$_1–2** and **MetH$^+$_4**, in which the carboxylic OH is not involved in any hydrogen bond interaction. This band is red shifted (1127 cm^{-1}) in structure **MetH$^+$_3** in which the carboxylic OH forms a hydrogen bond with the protonated ammonium group.

Five representative structures of different types of isomers of **[(MetSO)H$^+$]** are reported in Figure 3. Structures **MetH$^+$SO_1–3 and MetH$^+$SO_5** result from the addition of an oxygen on the sulphur atom and from the protonation of the amino group. Isomer **MetH$^+$SO_4** is characterised by the protonation of the sulfoxide group.

MetH$^+$SO_1 corresponds to the lowest energy structure on the **[(MetSO)H$^+$]** PES. It involves, like **[(Met)H$^+$]**, two strong intramolecular hydrogen bonds between the protonated amino group and the oxygen atoms of the sulfoxide and the carboxyl groups (NH···OS = 1.45 and NH···OC = 2.02 Å).

Structure **MetH$^+$SO_2** is 7.7 kJ/mol less stable than **MetH$^+$SO_1**. It is also characterised by two strong intramolecular hydrogen bonds: between the protonated amino group and the oxygen of the sulfoxide group, and between the protonated amino group and the oxygen atom of the OH group (NH···OS = 1.44 and NH···OH = 2.14 Å). If we assume a Maxwell–Boltzman distribution at room temperature, it should be populated only at 5% in our experimental conditions.

MetH$^+$SO_3, which is 14.52 kJ/mol less stable than **MetH$^+$SO_1**, is characterized by two strong hydrogen bonds between the protonated amino group and the oxygen atoms of the sulfoxide and carboxylic group, respectively (NH···OS = 1.54 and NH···OC = 2.34 Å). **MetH$^+$SO_3** differs from **MetH$^+$SO_1** in the value of the dihedral angle τ (–**C**H_2–**C**H_2–**C**H–**N**H_3–) which is of −69° for the former compared to −44° for the latter.

MetH$^+$SO_4, which result from the protonation on the sulfoxide group is 26.11 kJ/mol less stable than **MetH$^+$SO_1**. This structure is characterized by only one strong hydrogen bond between the S–OH group and the oxygen atom of the carboxylic group (SO–H···O=C=1.78 Å). **MetH$^+$SO_5**, which results again from the oxidation of the S atom, is 46.74 kJ/mol less stable than **MetH$^+$SO_1**. This structure is characterised again by two strong hydrogen bonds between the OH group and the oxygen atom of the sulfoxide group (O–H···O=S=1.52 Å) from one side and between the NH group of the amino group and the oxygen atom of the carboxylic group ((N–H···O=C=1.80 Å) on the other side.

A tentative assignment of the main IRMPD features of **[(MetSO)H$^+$]** is proposed in Table 2 by comparing the experimental vibrational frequencies with the ones calculated from the IR active vibrational modes of the lowest energy isomer **MetH$^+$SO_1**. The ratio between the experimental and the theoretical frequencies are also reported in the table.

The band at 1792 cm^{-1} (band A, Figure 3) in the experimental IRMPD spectrum of **[(MetSO)H$^+$]** can be assigned to the C=O stretching mode. The position of this band matches with the calculated band in structures **MetH$^+$SO_1**, **MetH$^+$SO_3 and MetH$^+$SO_5** in which the oxygen atom of the carbonyl group is involved in a hydrogen bond with the H-atom of the protonated amino group. The calculated C=O stretching mode is blue shifted in structures **MetH$^+$SO_2** (1850 cm^{-1}) because the carbonyl group is not involved in any hydrogen bond, while it is red shifted in structure **MetH$^+$SO_4** (1770 cm^{-1}) in which the carbonyl group is involved in a strong hydrogen bond with the S–OH group.

Table 2
Experimental and calculated vibrational frequencies for **[MetH$^+$SO]** lowest energy isomer, determined at the B3LYP 6-311+G** (5d, 7f) level of theory, and band assignments. The reported intensities given in parentheses are in km/mol.

Experiment (cm^{-1})	Calculation	Vibrational mode	Ratio (exp/theory)
947	931 (192)	νS=O	1.0
998	980 (72)	δCH_3	1.0
1162	1188 (201)	δCOH	0.98
1339	1346 (38)	δCH, δCH_2,δCOH	0.99
1420	1426 (40)	δCH, δCH_2,δCOH	0.99
1456	1463 (53)	δCH_2, δCH_3	0.99
1500	1583 (178)	$\delta_{umb}NH_3^+$	0.95
1534	1653 (79)	$\delta_{scissor}NH_3^+$	0.93
1792	1819 (256)	νC=O	0.99

Four bands are observed in the 1400–1600 cm^{-1} region in the experimental IRMPD spectrum (bands B–E, Figure 3). The assignment of these bands is more delicate. Two bands are present in the theoretical spectrum of **MetH$^+$SO_1** at 1653 and 1583 cm^{-1}. They may be attributed to the NH_3^+ scissoring and umbrella modes of the ammonium group, respectively. The calculated NH_3^+ umbrella mode for structure **MetH$^+$SO_1** (1583 cm^{-1}) is blue-shifted compared to that for **MetH$^+$_1** (1504 cm^{-1}). This is in agreement with the fact that the protonated amino group is involved in a stronger hydrogen bond with the sulfoxide group (NH···OS=1.45 Å) than in **MetH$^+$_1** (NH···S=2.13 Å). These two bands may be tentatively assigned to the experimental ones at 1534 and 1500 cm^{-1} (bands B and C, Figure 3), respectively. The NH_3^+ umbrella mode is red shifted in structure **MetH$^+$SO_3** (1492 cm^{-1}) where the amino group is involved in two less strong hydrogen bonds between the oxygen atom of the sulfoxide and the carbonyl group and in structure **MetH$^+$SO_5** (1427 cm^{-1}) where the amino group is involved in only one hydrogen bond.

Band D at 1456 cm^{-1} is attributed to the coupling of CH_2 and CH_3 bending modes, while the two bands at 1420 and 1339 cm^{-1} (bands E and F, respectively) can be attributed to the coupling of the bending mode of the C–OH group with those of the CH and CH_2 groups.

The intense band G at 1162 cm^{-1} may be assigned to the bending mode of the C–O–H group. The position of this band nicely

Table 3
Most intense peaks observed in the fragmentation mass spectra of the dipeptide and its oxidized form. A tentative assignment of the fragments is proposed in parenthesis.

Compound	*m*/*z*, *z* = +1
$[(Met–Lys)H]^+$	278 (parent ion)
Non-irradiated peptide	260 ($–H_2O$)
	243 ($–H_2O–NH_3$)
	215($–H_2O–NH_3–CO$)
	147 (y_1, protonated lysine) [36,37]
	129 ($y_1–H_2O$)
	84($y_1–H_2O–NH_3–CO$)
$[(MetSO–Lys)H]^+$	294 (parent ion)
Irradiated peptide with and without catalase	276 ($–H_2O$)
	259 ($–H_2O–NH_3$)
	230 ($–CH_3SOH$)
	212 ($–H_2O–CH_3SOH$)
	184($–H_2O–CH_3SOH–CO$ or $–H_2O–(CH_3–CH_2SO–CH_3)$)
	166 (Protonated methionine sulfoxide)
	147 (y_1, protonated lysine)
	129 ($y_1–H_2O$)
	84 ($y_1–H_2O–NH_3–CO$)

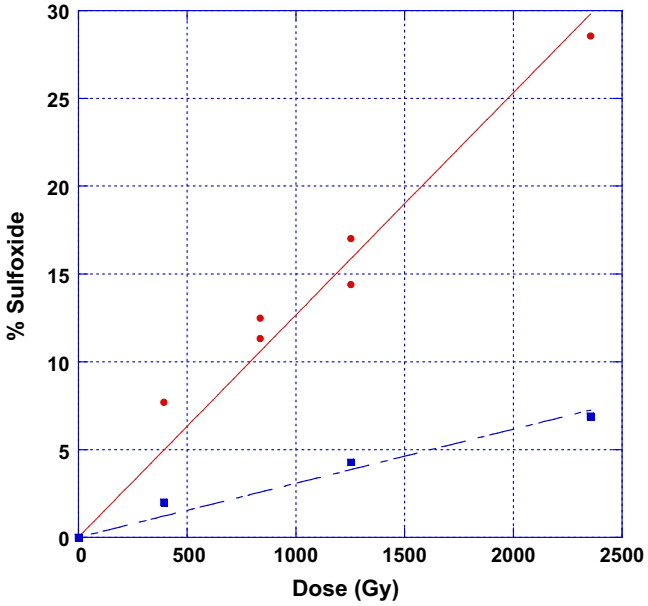

Figure 4. Relative intensity of the peak at m/z 294 as function of the irradiation dose (Gy) of an aqueous solution containing the dipeptide Met–Lys at pH 6 without catalase (red dots); with catalase present in the solution (blue squares) (circles).

matches that of the calculated one for structures **MetH$^+$SO_1**, **MetH$^+$SO_3** and **MetH$^+$SO_4**, in which the C–O–H group is not involved in any hydrogen bond. Its position is red shifted in the calculated spectrum of structure **MetH$^+$SO_2 and MetH$^+$SO_5**, in which the OH group is not free.

Band H at 947 cm^{-1} may be unambiguously attributed to the stretching mode of the S=O group, which forms a strong hydrogen bond with the protonated amino group. This band is in fact not observed at all in the IRMPD spectrum of [MetH$^+$] and can be used as a good diagnostic of the oxidation occurred on the sulphur atom.

The position of the band is, as a consequence of the strong hydrogen bond formed with the amino group, significantly red shifted compared to the stretching mode of the S=O group in model molecules like the dimethylsulfoxide [31] (webbook of chemistry) and the protonated sulphuric acid [32].

The position of the stretching mode of the S=O group in protonated sulphuric acid has been observed in the experimental IRMPD spectrum of the naked ion by Sinha et al. [32] at 1413 cm^{-1} in a good agreement with calculations. The stretching mode of the S=O group is calculated to be 1123 cm^{-1} for the neutral dimethylsulfoxide. This band constitutes an important vibrational signature of the presence of the S=O group and its position seems to depend on the surrounding environment.

3.3. Search for methionine sulfoxide among the products of oxidation of the dipeptide methionine–lysine by ·OH radicals

The dipeptide methionine–lysine (Met–Lys) was irradiated in aqueous solutions at pH 6 and 9.8 in N_2O atmosphere in the absence and in the presence of catalase. The non-irradiated sample (Table 3) presents the expected peak at *m/z* 278 (**[(Met–Lys)H]$^+$**)

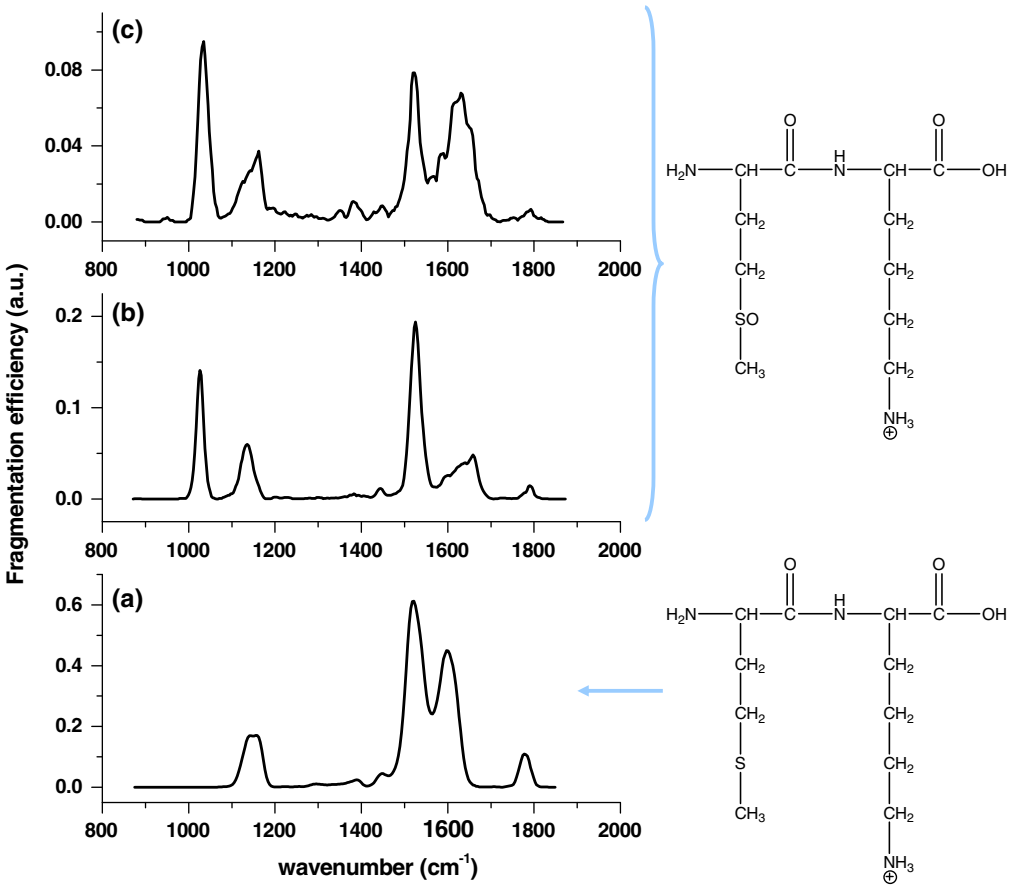

Figure 5. Comparison between the experimental IRMPD spectra of **[(Met–Lys)H$^+$]** (a), **[(MetSO–Lys)H$^+$]** without (b) and in presence of catalase (c) in the 800–2000 cm^{-1} energy range. The structures of the non-irradiated and irradiated protonated dipeptide Met–Lys are reported on the right.

(Supporting information Figure S2). In addition to this peak, the irradiated samples exhibit a peak at *m*/*z* 294, i.e. an addition of 16 to the initial compound (Table 3 and Figure S2). The relative intensity of this peak, with respect to that of the initial compound (*m*/*z* 278) increases linearly with the dose (Figure 4). This peak is higher if irradiation took place without catalase than in the presence of catalase, showing that a noticeable amount of this compound comes from the two-electron oxidation by hydrogen peroxide. Nevertheless, a substantial amount of (Met–Lys+16) is noticeable if irradiation took place with catalase.

The CID-MS^2 mass spectra of the dipeptides **[(Met–Lys)H]$^+$** non-oxidized and oxidized by ·OH radicals produced by water gamma radiolysis have been recorded. The *m*/*z* values of the most intense peaks present in the fragmentation mass spectra of the non-irradiated and irradiated dipeptides with or without catalase are reported in Table 3.

The non-irradiated samples behave quite similarly upon fragmentation at both pH values (6 or 9.8).

The IRMPD spectrum of **[(Met–Lys)H]$^+$** (Figure 5a) shows some similarity with that of protonated methionine (Figure 1) and some features resemble the IR spectrum of protonated L-lysine in the gas phase [33]. Four bands are observed. The band around 1200 cm^{-1} are reminiscent of that at 1170 cm^{-1} in **[MetH$^+$]** (Table 1 and Figure 1) and might be attributed to the bending mode of the C–O–H group. Those at 1500–1600 cm^{-1} should be due to the amine scissoring like in **[MetH$^+$]**.

In addition to these bands, **[(Met–Lys)OH]$^+$** exhibits a sharp band centred at 1050 cm^{-1} (Figure 5b and c) that falls in the range of the S=O bond stretching mode although it is blue-shifted compared to that of free methionine sulfoxide. The presence of this sharp band is in agreement with the formation of a sulfoxide function. This compound results not only from the oxidation by hydrogen peroxide, but also to the one-electron oxidation by ·OH radicals since it is still present with an unchanged shape if irradiation took place in the presence of catalase. We conclude that upon oxidation of the methionine residue in the dipeptide Met–Lys, methionine does lead to its sulfoxide and that the amount is proportional to the dose.

4. Conclusion

In this Letter we have unambiguously established the vibrational signature of the SO bond in methionine sulfoxide by IRMPD spectroscopy that exhibits an additional band compared to methionine in the 1000 cm^{-1} region. DFT calculations have allowed an interpretation of the spectrum and have confirmed that this band was due to the stretching mode of the SO bond.

We used this result to examine the problem of one-electron oxidation of a dipeptide containing methionine extracted from the human Prion protein sequence. These proteins are extremely rich in methionine and lysine residues (8 Met residues, 6 Lys residues in the 90–231 sequence) among them the Met–Lys sequence). The conversion of the cellular Prion protein (PrP(C)) into the infectious form (PrP(Sc)) is the key event in neurodegeneration [34]. The sulfoxidation of the methionine might be the switch for triggering the pathogenic conversion [35].

Our aim was also to unravel a controversy recently brought about [10] and to show whether or not **MetSO** was produced after one-electron oxidation of the methionine residue in the absence of oxygen. We used γ radiolysis in N_2O atmosphere to oxidize the peptide. ·OH radicals are the major oxidants and H_2O_2 is a minor compound (the yields are 0.55 and 0.07 $\mu mol\,J^{-1}$, respectively), thus the oxidants are the same as in oxidative stress. H_2O_2 was removed by the addition of catalase. The MS spectra of the Met–Lys peptide oxidized showed that the main oxidation product was the peptide to which an oxygen atom was added. IRMPD spectra unambiguously revealed the presence of a band characteristic of the SO bond elongation in the 1000 cm^{-1} region. Moreover, the addition of catalase prior to irradiation, which efficiently removed H_2O_2, did not suppress this peak. Its height was less, as expected. There is no doubt that the species having *m*/*z* 294, corresponding to the addition of oxygen to the protonated dipeptide (Met–Lys), exhibits a SO bond and thus is identified as the peptide having methionine sulfoxide.

As for the mechanism of sulfoxide formation in one-electron oxidation, the first steps are well known. The ·OH radicals add on the sulphur atom, which is followed by OH^- elimination. The sulfuranyl radical cation undergoes stabilization by the formation of a two centre-three electron bond with any atom having a lone pair doublet available ([7,8] and references therein). This stabilization notably increases the lifetime of the radical. We propose that this longer lifetime allows the radical to disproportionate, which would be accompanied by hydration of the sulphur. Our findings allow explaining why one finds methionine sulfoxide in proteins from the oxidation of methionine residue after oxidative stress.

Acknowledgments

Financial support by the European Commission to the EPITOPES project (Electron Plus Infrared TO probe and Elucidate Structures, EC project 15637) founded through the NEST (New and Emerging Science and Technology) program is gratefully acknowledged. We thank J.M. Ortega and the CLIO team for technical assistance. The very efficient assistance of V. Steinmetz during the IRMPD experiments is greatly acknowledged. We are indebted to the COST CM0603 (Free Radicals in Chemical Biology) for very fruitful discussions. We thank Prof. K.-D. Asmus for very stimulating discussions about the mechanism, and Prof. B. Marciniak for his support in this work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cplett.2010.12.012.

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