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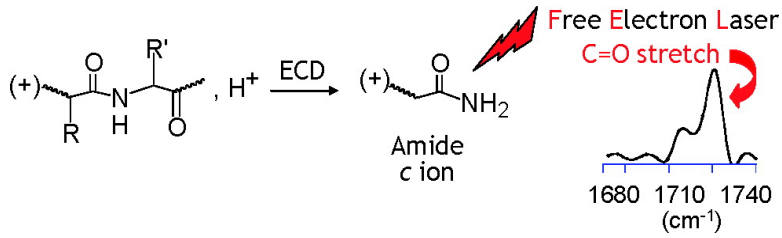

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Structure of Electron-Capture Dissociation Fragments from Charge-Tagged Peptides Probed by Tunable Infrared Multiple Photon Dissociation

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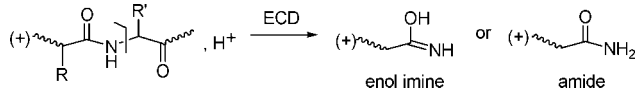
Electron capture dissociation (ECD) is an activation technique in Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry that has shown a promising potential for the analysis of peptides or proteins.[1] Electron capture by peptide or protein cations in the gas phase often results in extensive backbone fragmentation from which information about sequence as well as location of labile post-translational modifications can be obtained.[2] In ECD, multiply charged even-electron cations are partially reduced by receiving one electron and thus are converted to intermediate radical-cations which further fragment by backbone N−C_α bond cleavage to give *c*- and *z*-type ions.[3]

The structure of *c*-type ions formed by ECD and the overall mechanism leading to their formation are still a question of debate.[4] In the earliest mechanism proposed by McLafferty,[5] the electron attachment occurs at a protonated site that is H-bonded to a nearby carbonyl, to form a hypervalent radical. A hydrogen transfer from this radical to the carbonyl group induces a facile N−C_α bond cleavage and gives *c*-type ions that are proposed to have the enol-imine structure (Scheme 1). In the mechanism proposed by Turecek,[6] the electron capture occurs in a carbonyl π^* orbital and is followed, after proton transfer, by cleavage of the nearby N−C_α bond to give enol-imine *c*-type ions. A variation on this theme is the Utah−Washington (UW) mechanism,[6−8] in which the N−C_α bond is cleaved immediately after the electron capture in a carbonyl π^* and before any proton transfer. An interesting feature in this mechanism is its ability to directly give rise to amide structures that are more stable than their enol-imine counterparts (Scheme 1).

Scheme 1. Formation of *c*-Type Ions from a Doubly Charged Peptide



Since these *c*-type ions are isomeric, mass spectrometry alone cannot discriminate between them, but infrared spectroscopy can bring experimental evidence and help determine which scheme is operative.

IR spectroscopy recently proved to be a powerful tool to structurally characterize ions formed by collision induced dissociation (CID), showing for instance that oxazolone ring structures are formed for the b_4 CID fragment of Leu-enkephalin.[9] Here we show for the first time that infrared multiple photon dissociation (IRMPD) spectroscopy in the 1000−2000 cm^{-1} frequency range can be used to probe the structure of *c*-type ions formed by ECD. IRMPD spectra were obtained with the centre laser infrarouge d'Orsay (CLIO) IR free electron laser (FEL) coupled to a 7 T FT-ICR (APEX-Qe Bruker) mass spectrometer.[10]

In large systems such as peptides, the multiple C=O stretch modes often contribute to a spectral congestion that may restrict the structural information obtained from IRMPD spectroscopy. To overcome this limitation, a good candidate for the IR spectroscopy of *c*-type ions should be as vibrationally "silent" as possible in the C=O stretch region. Our first attempts at IRMPD of regular *c*-type fragment ions were unsuccessful. For c_1 ions, that are often of weak intensity, if not absent in the ECD spectra, we were unable to observe any dissociation with the FEL. For c_2 ions, the presence of two amide groups complicated the band assignment. In that context, we resorted to N-terminal derivatized peptides[11] which furnished an abundant c_0 ion upon ECD which could be further fragmented by the FEL.[12]

Suitable c_0 ions were obtained by ECD of $(TMPP\text{-}ac\text{-}GK+H)^{2+}$ where TMPP = tris(2,4,6-trimethoxyphenyl)phosphonium and ac = acetyl. These c_0 ions can have either the enol imine structure $TMPP^+-CH_2C(OH)=NH$ (**1**) or the amide one $TMPP^+-CH_2CONH_2$ (**2**) as shown in Scheme 1. Additionally, IRMPD spectrum of **2**, which is readily available as a byproduct of aminolysis of TMPP acetyl N-hydroxysuccinimidyl ester with ammonium bicarbonate buffer, was also recorded to provide a reference IRMPD spectrum for the amide form. Note that although the TMPP group has a large number (204) of vibrational modes, it has the advantage of being vibrationally silent in the C=O stretch region which is of prime importance for our experiments. Density functional theory (DFT) calculations were undertaken to obtain the most stable structures and theoretical IR spectra for both isomers.[13]

The most stable structures of **1** and **2** (Figures S1 and S2), and their theoretical IR spectra are represented in Figure 1. DFT calculations indicate that the relative energies of the amide and enol-imine isomers (~60 kJ mol^{-1} in favor of the amide form, see Table S1) are not substantially affected by the TMPP group. The calculated harmonic frequencies are much alike for **1** and **2** in the 1000−1650 cm^{-1} range due to a strong absorption of the TMPP group in this region. For instance, the most intense band at 1587 cm^{-1} corresponds to normal modes involving C−C stretches and C−H bends within the three aromatic rings. As expected, the signature bands for both isomers fall within the C=O stretch region. The amide isomer presents an absorption band at 1732 cm^{-1} assigned to the C=O stretch, whereas the enol-imine isomer, which has no C=O bond, presents a band at 1684 cm^{-1} assigned to the C=N stretch.

The experimental action spectra for c_0 ion and the amide reference **2** are very similar and close to the calculated absorption

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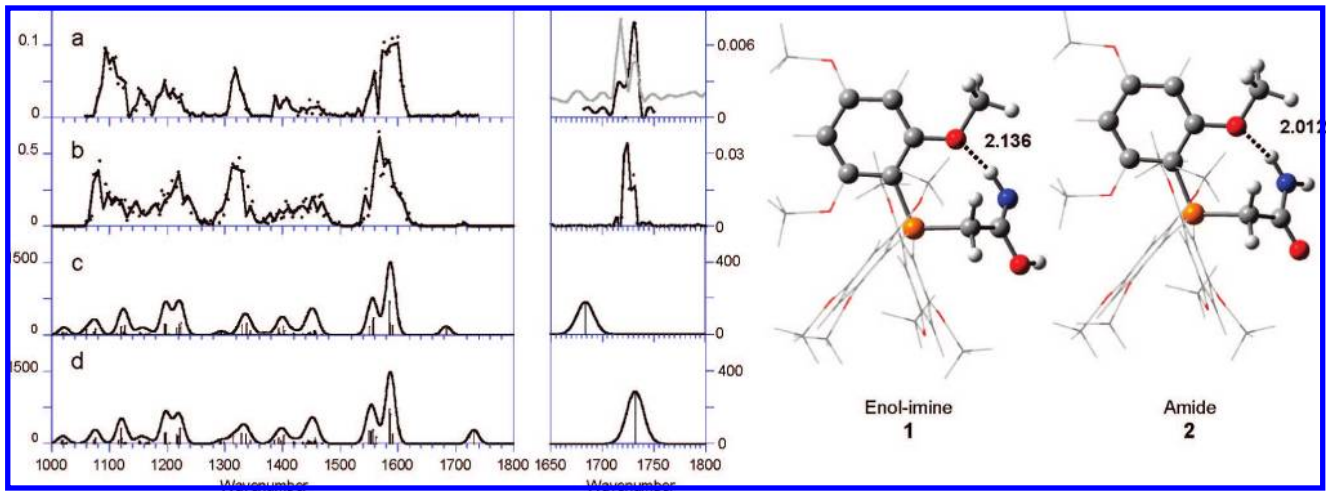

Figure 1. (Left panel) Experimental IRMPD action spectra of (a) the c_0 ion produced by ECD of $(TMPP\text{-}ac\text{-}GK+H)^{2+}$ and (b) the $TMPP\text{-}CH_2CONH_2$ reference compound. The vertical scale shows the fragmentation efficiency. Diamonds are experimental data points and the line is a cubic spline fit. B3LYP/6-31G(d,p) calculated absorption spectra for (c) enol-imine structure **1**, (d) amide structure **2**. Bars indicate the computed vibrational frequencies scaled by a factor of 0.96; the line shows an envelope of these frequencies after convolution with a 20 cm^{-1} fwhm Gaussian function. The vertical scale shows absorption in km mol^{-1}. (Middle panel) Experimental spectra in the 1650−1800 cm^{-1} region obtained in separate experiments after optimization of the lasing conditions to that specific region and increased spectra accumulation. For the c_0 ion, the IR spectra obtained from two different experiments are reported here: gray line, complete scan; black, rescan of the band at 1730 cm^{-1}. (Right panel) B3LYP/6-31G(d,p) optimized structures of most stable **1** and **2** conformers. For clarity, two of the trimethoxyphenyl group, as well as two of the methoxy group at the last phenyl group have been represented as sticks.

spectra in the 1000−1650 cm^{-1} range. After optimization of experimental conditions in the 1650−1800 cm^{-1} range, the action spectra exhibit one peak located at 1731 for c_0 and 1723 cm^{-1} for the amide reference. These values are similar within the experimental error and in close agreement with the 1732 cm^{-1} calculated for the amide isomer but distinct from the theoretical value for the enol-imine isomer (1684 cm^{-1}) to conclude that both the c_0 ion and the $TMPP\text{-}CH_2CONH_2$ reference have the amide structure. No indication of a band at 1680 cm^{-1} was found in the various spectra of the c_0 ion (Figure S4). Since the signal-to-noise ratio is rather poor, the presence of a minor fraction of enol-imine ions in the c_0 ion population cannot completely be ruled out.

Regarding the mechanisms that have been proposed for the formation of *c*-type ions upon ECD of peptides, those leading to enol-imine structures appear to be in contradiction with the present results. Do our data support the UW mechanism leading to the amide form? A definitive answer is not easy as one needs to take into account a possible isomerization of the enol-imine form to the more stable amide form subsequent to the cleavage of the $N-C_\alpha$ bond. An unassisted isomerization would involve a 1,3-H transfer of a high energy barrier which was computed to require 138 kJ mol^{-1} starting from **1** (Figure S3). Such an activation energy is typical for keto−enol isomerizations[14] and shows that the TMPP moiety does not internally catalyze the formation of the amide structure. However, unimolecular isomerization could occur in *c*-type ions of sufficient internal energy.[14] An energetically more favorable isomerization may take place in an intermediate long-lived ion−molecule complex. Proton migrations in such $c + z$ ion−molecule complexes have very low activation energies (3 kJ mol^{-1})[15] as do other catalyzed 1,3-H isomerizations.[14] A general rule for such catalysis is the presence of a base strong enough to abstract the proton and yield it back to the initial molecule. The lysine side chain would be a good candidate for such a process. Further work is in progress to draw a definite conclusion on this point.

In conclusion, we propose the first spectroscopic structural characterization of *c*-type ions produced by ECD of a peptide. IRMPD spectrum is assigned to an amide structure which is characterized by its IR signature at 1731 cm^{-1}. This result is particularly interesting for the elucidation of ECD mechanisms which continue to be the subject of an intense debate. However, additional experiments will be required to establish whether an isomerization from the enol-imine to the amide is involved in the formation of backbone peptide fragments.

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Supporting Information Available: Complete ref 13; computational and experimental details, Figures S1−S4 and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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