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Photon activation of peptides in the VUV

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Abstract. We present an overview on the photoactivation of gaseous peptide ions and point out the importance of synchrotron-based activation of peptides and proteins. Particularly, we present results on the action spectroscopy of substance P peptide in vacuum-ultraviolet (VUV) and discuss the importance of VUV-induced neutral losses over a wide photon energy range.

1. Introduction

Since the introduction of electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), mass spectrometry (MS) has been recognized as a powerful tool to study structure of biological molecules [1]. In biological analysis, the low sample consumption of MS and its ability to determine the structure of biomolecules is particularly appreciated. Very often, primary structures (which is the sequence of the constituting building blocks) of biological polymers are determined using tandem mass spectrometry (MS/MS), a particular arrangement in which a particular ion of interest is isolated, fragmented and the fragments mass analyzed. MS/MS has proven to be extremely useful for determination of the primary structures of biopolymers [1,2].

The usual activation methods are based on vibrational excitation of the target ions, such as low energy multiple collision-induced dissociation (CID) [3]. In the beginning of the 2000's, a technique based on the dissociative electron attachment to multiply protonated ions, and referred to as electron capture dissociation (ECD), has gained considerable attention in proteomic [4,5]. From a phenomenological point of view, ECD is able to fragment samples of high masses. It cleaves disulfide bridges and the protein backbones (mainly into c- and z- sequence ions) over an unusually high proportion of the amino acids, while retaining the labile bonds from post-translational modifications. There has been much debate concerning the nature and mechanism of ECD. It is now agreed that the electron attachment process releases a large amount of internal energy into the ion (~6 eV) through the formation of charge reduced species:

$$[M+nH]^{n+} + e^- \rightarrow [M+nH]^{(n-1)\bullet+} \quad (1)$$

The implementation of ECD is, however, restricted to Penning ion traps. In 2004 a variant of ECD appeared. It was based on electron transfer between anions and the targeted multiply protonated cation [6].

$$[M+nH]^{n+} + A^- \rightarrow [M+nH]^{(n-1)\bullet+} + A \quad (2)$$

This method referred to as Electron Transfer Dissociation (ETD), can be implemented on radiofrequency ion traps, which enlarge its field of application. Besides their large success, ETD and ECD still suffer from some limitations. Indeed, because these techniques cleave mainly the N-C_α bond, prolines do not fragment into *c*/*z*- ions owing to their cyclic arrangement. In addition, both ETD and ECD are not applicable to singly charged cations, as the electron transfer or capture would produce a neutral product, which is not detectable by the mass spectrometry.

Therefore, there was a clear need for new MS/MS methods able to produce different fragmentation patterns and providing at least partial control on the fragmentation channels. In that respect, absorption of UV photons is a straightforward mean to deliver rapidly a large amount of internal energy to an ion. This specificity explains the actual development in photon activation for tandem mass spectrometry.

2. Laser based photon activation

Photon activation of ions can be traced back to the 1960's-1970's with the pioneering work of Dehmelt and Jefferts [7], who reported the first photodissociation of molecular hydrogen cation, and that of R. C. Dunbar [8]. In these early experiments intense Xenon arc lamps were used in the visible and near ultra-violet with large band pass filters, as photoactivation requires powerful irradiations. However, in these early reports, photon activation was not applied to structural chemistry problems. In the early 1990's, McLafferty pioneered the field of laser photon activation of electrosprayed ions, through its study of biological molecules [9]. Large numbers of groups have worked on laser activation of ions, and these works cannot be reviewed here. Nowadays, MS is a corner stone of peptide and protein sequencing. The most widely used approach in proteomic relies on the study of mixture of peptides produced by the enzymatic digestion of the protein to be sequenced. The antagonistic approach deals with intact proteins, which are fragmented and analyzed in the gas phase by MS/MS. We will restrict our scope to peptides and proteins, owing to the importance of mass spectrometry in the field of proteomics. Activation of isolated ions in the gas phase is not trivial and requires very powerful light sources. Therefore, until recently, most of the studies have been performed using lasers.

2.1. UV spectroscopy and laser based-activation of peptides

The initial event of photon activation is the absorption of a photon by the target. Spectroscopy of peptides in the UV has been extensively studied, mainly because of the applications of circular dichroism for the determination of secondary structures. Briefly, two regions can be distinguished. Above 240 nm, most of the absorptions involves chromophores located on the side chains. For instance, aromatic side chains absorb in the 260-300 nm range, and disulfide show a weak band around 260 nm [10]. This regions have been probed for model protonated peptides in the gas phase by Perot *et al*. [11]. It appears that after 260 nm photon activation, the majority of the fragments involves side chain losses. Antoine and coworkers have compared the outcome of ECD, CID and laser activation for peptides at two different photon energies [12], and reported low abundant backbone fragment ions. Dugourd, Antoine and coworkers have investigated the 300-220 nm regions using tunable laser sources for peptides and proteins anions [13]. The main process upon irradiation of peptides anions is electron photodetachment, which could be related to electronic transitions involving the aromatic side chains [14]. Still, photon activation of anions produces fragments of low abundance [13].

Absorption below 240 nm is mainly due to $n\pi^*$ and $\pi\pi^*$ transitions [10] involving the peptidic bond. Spectroscopy of model peptides has been reviewed theoretically by Serrano-Andrès and Fülscher [15–18]. For small peptides, the $n\pi^*$ (W) transitions are found around 221 nm (5.6 eV) with low oscillator strength, followed by bright $\pi\pi^*$ (NV1) transitions at 200 nm (6.2 eV). However, a manifold of charge transfer (CT) excited states appear at a slightly shorter wavelength of188 nm (6.6

eV). Reilly and coworkers have probed the protonated peptides using excimer lasers at 193 (6.4 eV) and 157 nm (7.9 eV) [19,20]. These lines allows in principle to probe both NV1 and CT states. At both 193 and 157 nm, similar fragmentation patterns involving cleavage of the Cα-C bond and covering the entire backbone are observed. This series of so-called *a*- and *x*- sequence ions is accompanied by series of side chain losses into *d*- ions. For the model peptide substance P, results obtained by Reilly were in agreement with the pioneering report of Barbacci and Russel [21] and that of Yoon *et al*. [22]. However, these reports disagree on smaller abundance *c*- and *b*- ions.

Jennifer Brodbelt has established the 193 nm photon activation into a reliable and powerful method for proteomics. Extensive comparison with existing activation methods has shown that photodissociation increases the sequence coverage of entire proteins and also tryptic peptides [23] and is a valid alternative activation means for both top-down and bottom up proteomics.

2.2. Synchrotron radiation activation of peptides and action spectroscopy

Bari *et al*. [24] have first reported photon activation of a model peptide isolated in the gas phase using synchrotron radiation in the 8 to 40 eV range. They noticed that the fragmentation patterns changed for photon energy above the ionization threshold, although they couldn't observe the radical cation and thus measure the ionization energy of their protonated peptide. Other studies have shown that the protonated peptide radical was not stable enough to be detected [25–27]. This first report of action spectroscopy indicated that this spectroscopic technique could help to unravel open questions about the mechanisms occurring in laser photodissociation [24].

Later, we have investigated at several discrete photon energies the fragmentation patterns of a small human protein in association with its ligand [28]. We demonstrated for the first time that photodissociation preserved the non-covalent association of the ligand while the peptidic backbone was fragmented, thereby allowing the interaction site to be determined. Recently, Brodbelt and coworkers have characterized native protein complexes using photodissociation [29].

3. Action spectroscopy of substance P using synchrotron radiation

Our group has recently carried out a detailed study in the 5.5 to 20 eV for substance P [30,31]. As mentioned above, substance P has been probed using virtually any new activation method and especially using lasers. The spectroscopic region investigated was chosen so that it partly overlapped with the laser lines from previous works.

3.1. Photochemical reactions

The mechanisms leading to the wide coverage of the peptide and protein sequence is not established. Reilly and coworker proposed a Norrish type I reaction to account for the formation of *a*- and *x*- sequence ions [20]. Later, another mechanism involving Rydberg excitations has been proposed [32]. Thanks to the tunability of synchrotron radiation, we observed that the formation of *a*- ions in substance P over the 5.5-20 eV photon energy range was structured and exhibited feature that could be related to identified electronic excitations in polypeptides. Namely, the band observed for *a*- ions correlates well with the NV1 transitions. This observation pleads in favor of the Norrish type I reaction over the Rydberg hypothesis, owing to the discrete nature of these transitions. Moreover, a threshold was observed for *c*- fragments at higher energy. A Norrish type II transition occurring from the NV2 state has been tentatively proposed to account for the formation of *c*- ions [30].

3.2. Side chain losses versus backbone fragmentation

Side chain losses are not useful for protein sequencing. Large numbers of losses were reported for substance P and each of the partial ion yield exhibited features corresponding to the chromophores of the side chains. Figure 1 compares the ion yield for side chain losses normalized to both photon flux and ion current to the total fragmentation yields over the 5.5 to 18.5 eV photon energy range.

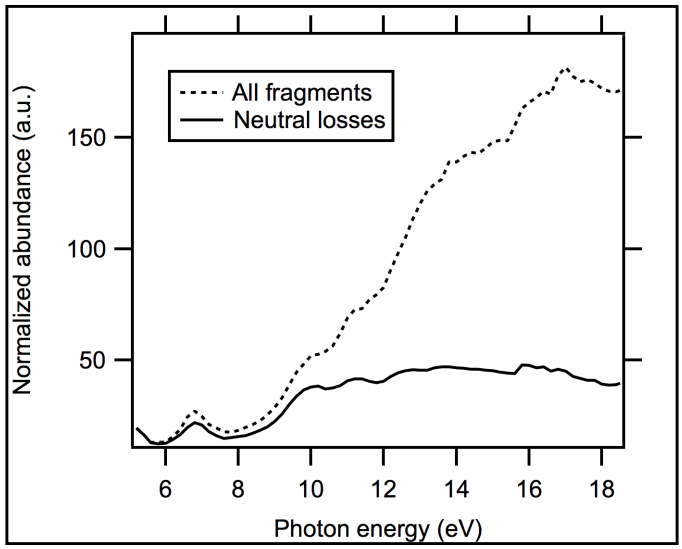

Figure 1. Comparison of the neutral losses observed from precursor with the total fragmentation yields.

It appears from figure 1 that for photon energy below the ionization threshold, the side chain losses represent the dominant dissociation channel; and particularly so below the threshold for backbone fragmentation reported (~6 eV) [30].

4. Conclusions

Action spectroscopy of peptide appears to be a valuable tool to study the photochemistry of biological species in the gas phase. Substance P has been shown to be an example of selective photochemistry. Moreover, the dominant dissociation channel below the ionization threshold is found to occur due to the side chain losses. This appears to be an interesting point considering the stability of biological molecules in general and of peptides in particular towards radiation damages. Indeed, side chain losses can be seen as mutations, since radiation changes the nature of the side chain and thus the nature of the amino acid. Therefore, the activity of the polypeptide may be perturbed. Still, these damages are less effective than those involving backbone cleavages. This point is the subject of an ongoing work.

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