Application of VUV synchrotron radiation to proteomic and analytical mass spectrometry

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Abstract. Recently, the coupling of ion traps with VUV beamlines has allowed performing activation of large biomolecular ions formed by electrospray. We discuss the potential of this new field along with the set up of an ion trap at the DESIRS beamline at SOLEIL. Study of primary structure of proteins becomes now possible at synchrotron facilities.

1. Introduction

Proteome is defined by the IUPAC as the complete set of proteins encoded by the genome [1]. The number of sequenced genome is increasing rapidly. However, it cannot provide information as to which proteins are expressed, to what extend, and how they are modified. Proteomic science is the quantitative or qualitative analysis of sets or subsets of proteins expressed by an organism under a given set of conditions [2].

Mass spectrometry is based on the analysis of gas phase ions sorted out as a function of their mass to charge ratio. The appearance of modern ionization techniques in the 1990’s has led to rapid development of mass spectrometry and separative methods in proteomics [3]. In some particular arrangements, the ability to isolate a specific m/z window, which corresponds to a compound of interest, either in space or time, allows performing tandem mass spectrometry [4,5]. Tandem mass spectrometry consists in first selection of an ion of interest, followed by an activation step leading to
an increase of the ion internal energy and ultimately to the fragmentation of the precursor. Proteins and poly-peptides fragment along a scheme, summarized in figure 1, which appears relatively simple regarding the chemical and structural complexity of these molecules [6]. The activation techniques of gas phase ions may be classified into two main groups. Usual activation methods are based on vibrational excitation of the target ions, such as low energy collision-induced dissociation (CID) or infrared multiphoton dissociation (IRMPD) [7], and lead to the cleavage of peptidic bonds with formation of \( b/y \)-sequence ions (figure 1). Recently, a technique based on the dissociative recombination of multiply protonated ions and referred to as electron capture dissociation (ECD), has gained considerable attention in proteomic [8] owing to some particular properties. 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 \( z \)-ions) over an unusually high proportion of amino acids, while retaining the labile bonds from post-translational modifications. Thus, the fragmentation pattern are complementary to those generated under CID conditions. There has been much debate concerning the mechanism of ECD [8], but it is admitted that the recombination releases large amount of internal energy into the ion (\( \sim 6 \) eV). However, as ECD cleaves N-C\( \alpha \) bond, proline amino acids preclude fragmentation into \( c/z \)-ions due to their cyclic arrangement.

![Figure 1. Nomenclature for peptide backbone fragmentation.](image)

The ability to identify proteins from mixture as well as their post-translational modifications using tandem mass spectrometry has deeply affected the existing protein sequencing paradigm and launched a new strategy called shotgun proteomics. The most classical strategy to analyze the proteome, know as “bottom-up”, is based on the identification of protein characteristic peptides. It involves producing peptide fragments from an enzymatic digestion of the proteins of interest. Generally bottom-up approaches rely on either the mass measurement of the set peptide obtained from the digestion giving (peptide mass fingerprinting) or on fragmentation of several of these peptides in MS/MS experiments. The bottom-up strategy barely provides full sequence coverage, which limits its applicability to site-specific mutations and identification of post-translational modifications. An antagonist approach, referred to as “top-down”, involves fragmentation of intact protein in the gas phase. In general, slow heating methods (CID, IRMPD…) lead to the loss of post-translational modifications, because modifications are the most labile and fragile bonding on the protein [9]. ECD has been shown to retain
the modifications, thus allowing identification of the site of the modification [9]. However, ECD has some limitations, such as the necessity to work on multiply protonated precursor ions (owing to the charge reduction) and low fragmentation efficiencies.

Therefore, there are real needs in this field for new activation methods able to cleave large ions, with high efficiency and able to retain non-covalent interactions and posttranslational modifications. As discussed above, the heart of tandem mass spectrometry technologies is to increase the amount of internal energy of an ion of interest. At first sight, a simple and straightforward way to achieve this would rely on photoabsorption. Indeed, the absorption of photons in the VUV domain would provide several eV to the target, populating electronic excited states whose fragmentation decay properties might reveal desirable.

2. Experimental set-up

Earlier set-ups for ion spectroscopy at synchrotron facility were based on merged beam experiments in which ion beams of high current were merged over tenth of centimeter and up to one meter with monochromatic synchrotron radiation [10]. Excellent sensitivity has been achieved and cross sections of below the Mbarn are within reach. However, the ion sources used in these set up are not compatible with biological molecules. The ion trap based experiments compensate for the lower ionic current and smaller overlap with longer irradiation time. The feasibility of performing ion spectroscopy in ion traps has been demonstrated on a Penning trap at Elettra [11]. Linear ion traps have been shown to give access to a few tenth of Mbarn for 1 s irradiation [12].

A project called Synchrotron Radiation for Tandem Mass Spectrometry (SRMS2) has been launched to evaluate the feasibility and the practical aspects of performing ion activation in the VUV at the SOLEIL synchrotron radiation facility. A linear ion trap (LTQ XL) from Thermo Scientific has been coupled to the DESIRS beamline [13], as illustrated in figure 2. LTQ mass spectrometers offer excellent instrumental capabilities, such as 50 ppm mass accuracy, attomole sensitivity (on reserpine), 15-4000 m/z range, resolving power above 25 000 (FWHM), fairly high scan rates (16 000 units/s), and compatibility with various ions sources (ESI, nano, APPI, APCI…), which makes it a versatile instrument suited for proteomic. Beside these qualities, the linear trap is directly accessible when a plate is removed from the back of the instrument, which allows easy irradiation of the stored ion. A homemade plate fitted with DN40CF connection has replaced the original one. A differential pumping stage accommodates for the pressure difference between the ion trap (10^{-5} mbar) and the beamline (10^{-9} mbar). An electromechanical photon shutter, placed just upstream the mass spectrometer, opens during the tandem mass spectrometry sequence and let the photons in.
However, the coupling of an ion trap with a synchrotron radiation beamline to perform ion irradiation is not a simple task. Indeed, the ions packet of 2 mm in diameter by 30 mm in length containing up to $3 \times 10^4$ ions has to be irradiated for enough time with sufficient photon fluxes.

The low resolution high flux 200 grooves/mm grating of DESIRS was used, with the entrance and exit slit open a 200 and 400 µm, respectively, giving typically 12 meV resolution and 10 eV. Typical irradiation times are around 200 ms at DESIRS for $10^{12}$ photons/s fluxes.

### 3. Results and applications

When positive ions are submitted to UV photons, clearly two photon-energy regimes may be distinguished. Below the ionization threshold, the fragmentation regime is based on photodissociation. Laser based experiments have shown that irradiation at 193 nm and 157 nm produces $a$- and $x$-sequence ions [14], which is totally complementary to the classical CID and ECD methods. Experiments using VUV synchrotron radiation confirms that below the ionization threshold mainly $a/x$- species are formed [15]. The ionic abundances have been reported to increase with the photon energy, which is consistent with the shape of the photoabsorption cross section of the proteins. When the photon energy is increased above the ionization threshold of the ion, an electron may be ejected producing a radical cation. In this dissociative photoionization regime, $b/y$- and $c/z$- fragment ions appear. The variety and the abundance of fragment is more important than below the threshold. The optimum appears around 18 eV, certainly because the photoabsorption cross-section is maximum at this photon energy.
4. Conclusions

Besides macromolecular crystallography, study of secondary structure of proteins was possible at synchrotron radiation facilities on circular dichroism end-stations. Ternary and higher order structures are accessible on X-rays scattering beamlines. But hitherto, access to primary structure was not granted at SR facilities. New developments in coupling ion traps with VUV beamlines offer applications in mass spectrometry based proteomic. Indeed, VUV activation is a new activation methods, complementary to existing one with promising potential.

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