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VUV synchrotron radiation: a new activation technique for tandem mass spectrometry

Aleksandar R. Milosavljević,[a,b] Christophe Nicolas,[a] Jean-Francois Gil,[a] Francis Canon,[a] Matthieu Réfrégiers,[a] Laurent Nahon[a] and Alexandre Giuliani[a,c]*

[a]Synchrotron SOLEIL, L'Orme des Merisiers, 91192 Gif sur Yvette Cedex, France, [b]Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia, and [c]INRA, U1008 CEPIA, Rue de la Géraudière, F-44316 Nantes, France. E-mail: alexandre.giuliani@synchrotron-soleil.fr

A novel experimental technique for tandem mass spectrometry and ion spectroscopy of electrosprayed ions using vacuum-ultraviolet (VUV) synchrotron radiation is presented. Photon activation of trapped precursor ions has been performed by coupling a commercial linear quadrupole ion trap (Thermo scientific LTQ XL), equipped with the electrosprayed ions source, to the DESIRS beamline at the SOLEIL synchrotron radiation facility. The obtained results include, for the first time on biopolymers, photodetachment spectroscopy using monochromated synchrotron radiation of multi-charged anions and the single photon ionization of large charge-selected polycations. The high efficiency and signal-to-noise ratio achieved by the present set-up open up possibilities of using synchrotron light as a new controllable activation method in tandem mass spectrometry of biopolymers and VUV-photon spectroscopy of large biological ions.

Keywords: mass spectrometry; tandem mass spectrometry; ion; spectroscopy; VUV; activation; biopolymer; sequencing.

1. Introduction

Mass spectrometry (MS) is based upon the analysis of gas-phase ions mass-to-charge ratio (m/z), whose distribution produces a mass spectrum. Since the introduction of electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), mass spectrometry has been recognized as a powerful tool for the study of the structures of biological molecules (Biemann, 2002). Particular instrumental arrangements offer the possibility of isolating a specific m/z window either in time or in space, leading to so-called tandem mass spectrometry capabilities. Tandem mass spectrometry, also referred to as MS/MS or MS^2, can be briefly described as the following sequence of events (Dass, 2001; de Hoffmann, 1996). First, a selected m/z window, corresponding to the targeted precursor ion, is isolated. In a second step, those precursor ions are activated, meaning that their internal energy is increased which eventually leads to the fragmentation of the precursor. Usually, this process, termed collision-induced dissociation (CID; Haddon & McLafferty, 1968; Rozett & Koski, 1968), is carried out by inelastic collisions with a gas, such as helium or nitrogen. The product ions are finally analyzed according to their m/z giving a tandem mass spectrum. Note that the previous isolation, activation and fragmentation procedure can be repeated further up to the nth level (MS^n). The goal of tandem mass spectrometry is to provide detailed structural information on a particular species of interest through successive fragmentation. In particular, tandem mass spectrometry has proven extremely useful for determination of the primary structure of biopolymers (Biemann, 2002; de Hoffmann, 2001). However, the crucial point in the application of the MS^2 technique to the study of large biopolymers is the need for an efficient activation method, which can produce an intense and selective fragmentation of such species.

The aim of this paper is to show that vacuum-ultraviolet (VUV) synchrotron radiation may be used as an efficient and precise activation technique for tandem mass spectrometry, with several important advantages over classical methods.

Conventional activation methods in MS^2 are based upon the vibrational excitation of the precursor ion, which can be performed *via* CID or infrared multiphoton dissociation (Little *et al.*, 1994; Price *et al.*, 1996). However, those slow heating activation techniques still impose some important limitations. Firstly, since the energy required for an ion to dissociate increases with its size, these methods may fail to efficiently cleave large biomolecules (Dass, 2001). Furthermore, dissociations by slow heating do not preserve non-covalent associations forming larger assemblies. In recent years, several new techniques allowing improved MS^2 have emerged, such as electron capture dissociation (ECD) (Zubarev *et al.*, 1998), electron ionization dissociation (Fung *et al.*, 2009) and femtosecond laser-based ionization/dissociation (Kalcic *et al.*, 2009). However, they still have some limitations

(such as low efficiency for dications and the impossibility to apply it on singly charged ions for ECD) or they need to be improved in terms of analytical capabilities. Therefore, there is a clear need in MS^2 biological analysis for alternative modes of ion activations, which can improve the efficiency of the internal energy deposition and provide selective fast fragmentation.

A simple and straightforward means of depositing a large amount of internal energy into the ions of interest is absorption of energetic photons by the target, typically in the VUV range. Indeed the absorption of a single photon in this energy domain is enough to activate a given precursor ion by a 'clean' fragmentation/ionization (or photodetachment in the case of anions) process. The first advantage of such VUV-based activation techniques over previous ones would be the large quantity of analytical information (intensive and clean fragmentation), even for biological species where modern and usually very efficient activation methods such as ECD do not perform well (*e.g.* prolines). In addition, a very important advantage of the present method is the photon tunability, allowing the activation to be controlled by accessing different fragmentation/ionization limits. As a consequence, it might be possible for certain photon energy to localize specific structural properties of biomolecules (*e.g.* sites of non-covalent interaction). In that sense, tunability is of course mandatory not only for MS^2 analytical applications but also for opening a large alley of research on the VUV spectroscopy of gas-phase biopolymer ions.

However, coupling an ion trap to the synchrotron beam is not a trivial task because of difficulties in achieving high enough ion densities and photon fluxes, even though the signal-to-noise ratio benefits from the possibility of performing longer irradiation of the trapped ionic species. The present linear ion trap spectrometer (LTQ XL) can confine large ionic densities with limited space charge using a helium bath. On the other hand, intense laser sources are unable to provide high-energy photons in a large tunable domain, while the conventional UV lamps are not brilliant enough for the purpose. Therefore, the only light source that can deliver sufficient photon fluxes over a wide energy range is synchrotron radiation, especially on third-generation sources, offering both an intrinsic tunability and a high brilliance. To our knowledge, a first attempt of coupling a Paul trap to synchrotron radiation was reported some years ago (Kravis *et al.*, 1991); however, the limited success of the experiment came from their use of the white-beam emission from a bending magnet. The feasibility of coupling a Fourier-transform ion cyclotron resonance ion trap with a soft X-ray synchrotron radiation beamline has been demonstrated very recently by Thissen *et al.* (2008) and by Hirsch *et al.* (2009) for the coupling of a linear ion trap for the study of gas-phase atomic species and clusters. The first successful use of non-dispersed undulator radiation for spectroscopy of electrosprayed negative ions stored in a three-dimensional quadrupole ion trap, in the 290–80 nm wavelength range (4.3–15.5 eV), was preliminary reported only in 2009 (Giulliani, 2009). Still, it should be noted that both reported experiments (Thissen *et al.*, 2008; Kravis *et al.*, 1991) suffered from a rather low signal-to-noise ratio. Note that, very recently, Bari *et al.* (2011) have reported the coupling of a Paul trap with a synchrotron radiation beamline at the BESSY synchrotron facility.

In the present communication we report on a novel experimental set-up, coupling a linear quadrupole ion trap equipped with an ESI source to a third-generation synchrotron undulator-based VUV beamline. Our set-up shows the possibility of using synchrotron-radiation-induced activation as a new complementary MS^2 technique as well as it providing an efficient VUV spectroscopy technique for electrosprayed biopolymer ions isolated in the gas phase. The system involves several important advantages and improvements leading to an excellent signal-to-noise ratio for an ion spectroscopy and activation set-up.

2. Experimental set-up

A schematic drawing of the experimental assembly is given in Fig. 1. It is based upon a commercial linear quadrupole ion trap mass spectrometer (LTQ XL; Thermo Fisher), equipped with an ESI source. The electrosprayed ions are introduced from the front side of the spectrometer and guided throughout the system into a linear quadrupole ion trap (Schwartz *et al.*, 2002). Mass analysis is performed by ejecting ions out of a slot in two opposite rods using the mass selective instability mode of operation. The synchrotron beam is sent directly into the trap through the back lens of the quadrupole ion trap from the

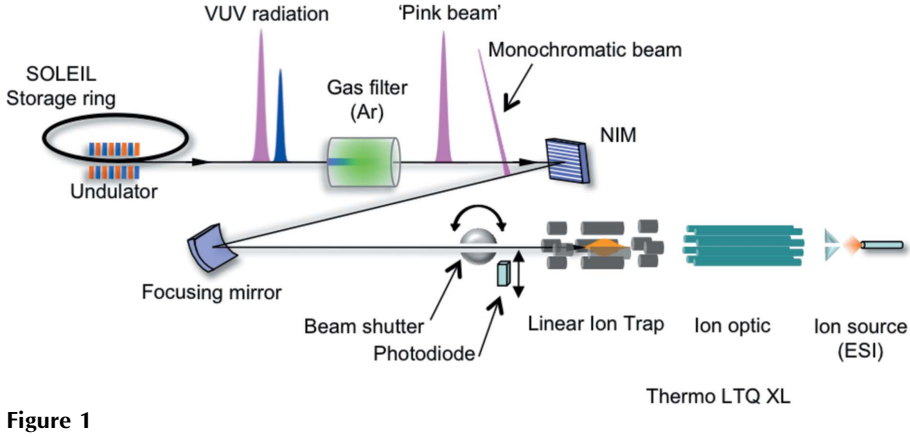

Figure 1
Schematic of the coupling of the linear ion trap with the DESIRS beamline of the SOLEIL synchrotron radiation facility. VUV produced by the OPHELIE2 (HU640 type) electromagnetic undulator of the beamline is filtered by a gas filter, which removes the harmonic content of the source spectrum. The so-called 'pink beam' with 7% bandwidth may either be sent to the experiment with the monochromator set at the zero order or it may be monochromated by the normal-incidence monochromator (NIM). Downstream, the beam is deflected and refocused by a mirror to the user port. The ion trap is mounted on the post-focusing arm port. A beam shutter is used to allow the incoming VUV light inside the trap for some controlled duration.

back of the spectrometer. To achieve this, the original back plate of the spectrometer has been replaced by a home-made plate fitted with a flange to connect with the differential pumping stage (turbomolecular pump of 300 l s^{-1}) that accommodates the pressure difference between the spectrometer (10^{-5} mbar) and the beamline port (10^{-8} mbar). The pumping stage includes a retractable calibrated photodiode (AXUV100; International Radiation Detectors) for photon flux measurements and a photon beam shutter (Milosavljević *et al.*, 2011*a*). The latter has been especially designed to provide a short rise time ($\simeq$ 1 ms) and reproducible chopping operation under high-vacuum conditions.

The main difficulties in photo-excitation of ions are: (i) achieving a high enough ion density for a sufficient long period of time, (ii) sufficient overlap between the ion beam and the synchrotron radiation beam, (iii) sufficient photon flux, (iv) a long enough interaction path length and (v) enough detection efficiency for ions. The product ion count rate may be estimated by the following formula (West, 2001; Hirsch *et al.*, 2009): $S = FIJL\sigma$, where S is the measured ionic signal per second, F is the form factor describing the overlap between the two beams, I is the photon flux (photons s^{-1}), J is the ion density (ions cm^{-3}), L is the interaction path length (cm) and σ is the cross section of the process. The ionic trapping cylindrical volume of length 30 mm and diameter 2 mm is formed by four hyperbolic rods. The LTQ XL mass spectrometer may confine up to 3×10^4 ions within that trapping volume (10^6 ions cm^{-3}) without space charge. Hence, for irradiation times of 1 s, processes with cross sections as low as a tenth of a Mbarn may be observed with photon fluxes of 10^{12} photons s^{-1}. Hence, a high photon flux and optimal overlap between the incident photons and trapped ion packet is crucial to achieve a good signal-to-noise ratio. Finally, tunability over a wide wavelength range in the VUV is mandatory for obtaining selectivity in the fragmentation processes. These constraints on the photon source are only met at third-generation synchrotron radiation sources, especially on undulator-based beamlines.

The experimental set-up has been installed as a non-permanent endstation on the VUV beamline DESIRS (http://www.synchrotron-soleil.fr/portal/page/portal/Recherche/LignesLumiere/DESIRS; Nahon *et al.*, 2012) at the SOLEIL storage ring in St Aubin (France). The electromagnetic undulator OPHELIE2 (HU640 type) provides tunable radiation in the 5–40 eV energy range (Marcouille *et al.*, 2007). The wavelength is selected by a 6.65 m-long normal-incidence monochromator (Nahon *et al.*, 2001) equipped with a low-dispersion (200 grooves mm^{-1}) grating providing a high photon flux of the order of 10^{12}–10^{13} photons s^{-1} over the energy range, within a photon bandwidth of typically 12 meV at 10 eV photon energy with typically a 200 µm exit slit. Higher harmonics of the undulator are cut off by a gas filter filled with Ar or Xe (Mercier *et al.*, 2000) or at low energies by MgF_2 or SUPRASIL windows.

The experimental procedure consists of the following sequence of events, for a given wavelength: (i) electrosprayed ions are injected, selected and stored in the trap; (ii) when the desired ion capacity is reached, the beam shutter opens, thus starting the irradiation; (iii) after the desired time of irradiation, the shutter intercepts the beam; (iv) the mass spectrum is recorded; (v) the monochromator and the position of the undulator maximum are set to the next wavelength, in the so-called gap-scan mode, and the procedure is repeated. The irradiation time is regulated directly by the LTQ XL that masters opening/closing of the photon shutter. The automatic energy scanning and mass spectra recording has been achieved by use of a separate personal computer and a home-made program, which synchronizes the operation of both the beamline and the mass spectrometer.

3. Results

A model protein, the cytochrome *c* (104 residues, 12384 Da), was submitted to the experimental procedure previously described. Fig. 2 presents a typical photon activation MS^2 spectrum of the 4^+ precursor of cytochrome *c* (m/z = 3090), recorded after about 600 ms of irradiation at a photon energy of 14.25 eV. This spectrum reveals a very efficient ionization of polyprotonated cytochrome *c* protein upon absorption of a VUV photon. Moreover, following the dominant ionized 5^+ species at m/z = 2477.6, a series of features corresponding to 6^+, 7^+ and 8^+ ionized precursor can be observed in the spectrum. The multiply ionized molecules are most probably formed by a successive single photoionization, as their intensity linearly decreases with decreasing irradiation time. A more detailed discussion on the photoionization efficiency curves and fragmentation patterns exceeds the scope of the present communication and is partly the subject of a separate publication (Milosavljević *et al.*, 2011*b*). Nevertheless, it is important to point out that VUV synchrotron radiation appears to be a very efficient activation method for the trapped protein precursor ions. Note that the MS^2 spectrum recorded under the same conditions without the photon beam

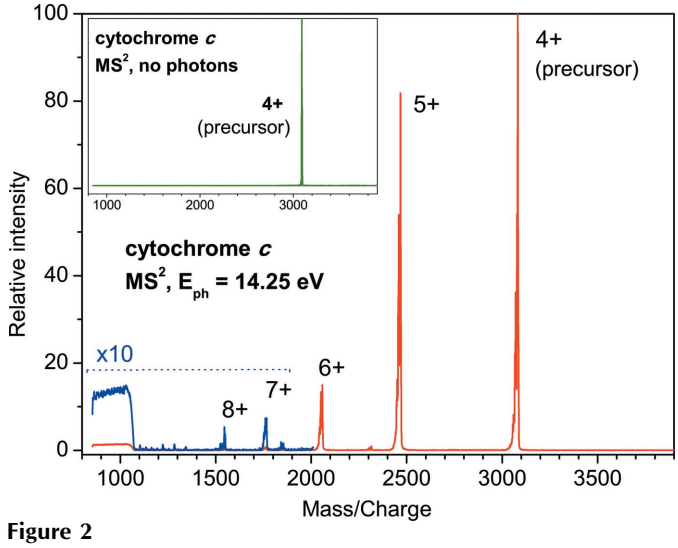

Figure 2
Mass spectrum of the product ion resulting from photoionization of the 4^+ precursor cytochrome *c* ion at 14.2 eV. The precursor ion was isolated, stored in the ion trap and irradiated for 600 ms.

does not exhibit, as expected, any additional features to the precursor peak (inset of Fig. 2). Furthermore, the signal-to-noise ratio is high enough to resolve all features in the mass spectrum, even in the monochromated undulator radiation, while a noticeable noise appears only at low m/z values. It should also be noted that the noise further decreases with decreasing irradiation time and photon energy.

Fig. 3 shows a photon activation MS^2 spectrum of the negative 6^- precursor of cytochrome *c* molecule (m/z = 2063), recorded after about 200 ms of irradiation at a photon energy of 15.0 eV. The dominant channel at m/z = 2475.6 corresponds to the formation of the radical species $5^{-\bullet}$, which is formed by electron photodetachment from the precursor ion. Additionally, the feature corresponding to double photodetachment (m/z = 3083) is clearly resolved in the spectrum. It should be noted that electron detachment from trapped peptide and protein polyanions *in vacuo* by ultraviolet/visible (UV/Vis) laser irradiation has been recently studied (Joly *et al.*, 2007; Brunet *et al.*, 2011). The proposed mechanism involves an electronic excitation of the chromosphere state leading to the electron detachment *via* a crossing with an auto-ionizing state of the molecule. As a consequence, photodetachment spectroscopy can be used to probe the electronic structure of a complex biomolecule isolated in the gas phase. The first VUV photodetachment study (above about 4 eV of photon energy) of electrosprayed peptide ions, and only conducted with non-dispersed undulator radiation light, has been preliminary reported recently (Giulliani, 2009). The present results demonstrate that photodetachment spectroscopy of a 12.4 kDa cytochrome *c* protein precursor can be efficiently performed using a VUV synchrotron radiation source.

4. Conclusion

The present paper reports on a novel experimental system that uses VUV synchrotron radiation as an activation method for quadrupole ion trap tandem mass spectrometry. The achievements of the present system are the first photodetachment spectroscopy of electrosprayed biopolymers using monochromated synchrotron radiation, as well as the first ionization spectroscopy study of large polycations. More than satisfactory performances and sensitivity of the present experimental set-up provide the possibility to study fundamental physical and structural properties of large biological ions isolated in the gas phase by use of synchrotron-radiation-based VUV spectroscopy. Furthermore, the efficient formation of radical species upon photoionization of trapped ions paves the way for the development of a new complementary tandem mass spectrometry technique, which uses VUV synchrotron radiation as a controllable soft-ionization activation method. Besides existing and well established methods such as macromolecular crystallography, small- and wide-angle X-ray scattering or synchrotron radiation circular dichroism, our technique appears then as a novel and complementary method for studying the structure and physical properties of biopolymers using synchrotron radiation.

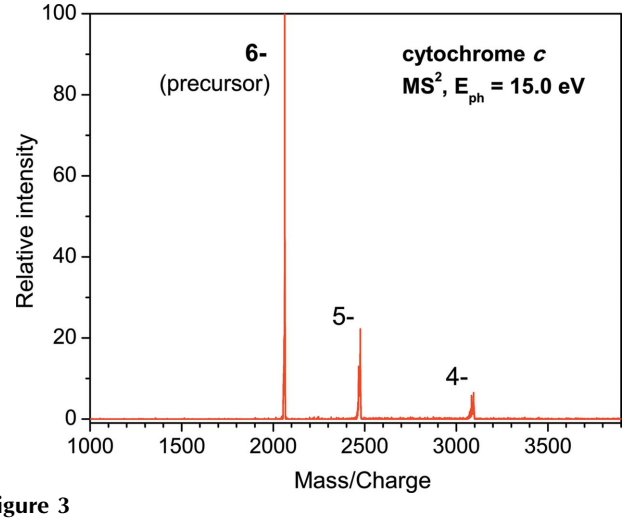

Figure 3
Mass spectrum of the product ion resulting from photodetachment of the 6^- precursor cytochrome *c* ion at 15 eV. The precursor ion was isolated, stored in the ion trap and irradiated for 200 ms.

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References

Bari, S., Gonzalez-Magaña, O., Reitsma, G., Werner, J., Schippers, S., Hoekstra, R. & Schlathölter, T. (2011). *J. Chem. Phys.* **134**, 024314.
Biemann, K. (2002). *J. Am. Soc. Mass Spectrom.* **13**, 1254–1272.
Brunet, C., Antoine, R., Allouche, A. R., Dugourd, P., Canon, F., Giuliani, A. & Nahon, L. (2011). *J. Phys. Chem. A*, **115**, 8933–8939.
Dass, C. (2001). *Principles and Practice of Biological Mass Spectrometry*. New York: John Wiley and Sons.
Fung, Y. M., Adams, C. M. & Zubarev, R. A. (2009). *J. Am. Chem. Soc.* **131**, 9977–9985.
Giulliani, A. *et al.* (2009). *Proceedings of the 57th ASMS Conference on Mass Spectrometry and Allied Topics*, Philadelphia, PA, USA, 31 May–5 June 2009.
Haddon, W. F. & McLafferty, F. W. (1968). *J. Am. Chem. Soc.* **90**, 4745–4746.
Hirsch, K., Lau, J., Klar, P., Langenberg, A., Probst, J., Rittmann, J., Vogel, M., Zamudio-Bayer, V., Möller, T. & Issendorff, B. (2009). *J. Phys. B*, **42**, 154029.
Hoffmann, E. de (1996). *J. Mass Spectrom.* **31**, 129–137.
Hoffmann, E. de (2001). *Mass Spectrometry. Principle and Applications.* New York: Wiley.
Joly, L., Antoine, R., Allouche, A. R., Broyer, M., Lemoine, J. & Dugourd, P. (2007). *J. Am. Chem. Soc.* **129**, 8428–8429.
Kalcic, C. L., Gunaratne, T. C., Jones, A. D., Dantus, M. & Reid, G. E. (2009). *J. Am. Chem. Soc.* **131**, 940–942.

Kravis, S. D., Church, D. A., Johnson, B. M., Meron, M., Jones, K. W., Levin, J., Sellin, I. A., Azuma, Y., Mansour, N. B., Berry, H. G. & Druetta, M. (1991). *Phys. Rev. Lett.* **66**, 2956–2959.
Little, D. P., Speir, J. P., Senko, M. W., O'Connor, P. B. & McLafferty, F. W. (1994). *Anal. Chem.* **66**, 2809–2815.
Marcouille, O., Brunelle, P., Chubar, O., Marteau, F., Massal, M., Nahon, L., Tavakoli, K., Veteran, J. & Filhol, J. M. (2007). *AIP Conf. Proc.* **879**, 311–314.
Mercier, B., Compin, M., Prevost, C., Bellec, G., Thissen, R., Dutuit, O. & Nahon, L. (2000). *J. Vac. Sci. Technol. A*, **18**, 2533–2541.
Milosavlejić, A. R., Nicolas, C., Gil, J.-F., Canon, F., RéFrégiers, M., Nahon, L. & Giuliani, A. (2011*a*). *Nucl. Instrum. Methods Phys. Res. B*, doi:10.1016/j.nimb.2011.10.032.
Milosavlejić, A. R., Nicolas, C., Lemaire, J., Déhon, C., Thissen, R., Bizau, J.-M., RéFrégiers, M., Nahon, L. & Giuliani, A. (2011*b*). *Phys. Chem. Chem. Phys.* **13**, 15432–15436.
Nahon, L., Alcaraz, C., Marlats, J. L., Lagarde, B., Polack, F., Thissen, R., Lepere, D. & Ito, K. (2001). *Rev. Sci. Instrum.* **72**, 1320–1329.
Nahon, L., de Oliveira, N., Garcia, G. A., Gil, J.-F., Pilette, B., Marcouillé, O., Lagarde, B. & Polack, F. (2012). *J Synchrotron Rad.* In preparation.
Price, W. D., Schnier, P. D. & Williams, E. R. (1996). *Anal. Chem.* **68**, 859–866.
Rozett, R. W. & Koski, W. S. (1968). *J. Chem. Phys.* **49**, 2691–2695.
Schwartz, J. C., Senko, M. W. & Syka, J. E. (2002). *J. Am. Soc. Mass Spectrom.* **13**, 659–669.
Thissen, R., Bizau, J. M., Blancard, C., Coreno, M., Dehon, C., Franceschi, P., Giuliani, A., Lemaire, J. & Nicolas, C. (2008). *Phys. Rev. Lett.* **100**, 223001.
West, J. B. (2001). *J. Phys. B*, **34**, R45.
Zubarev, R. A., Kelleher, N. L. & McLafferty, F. W. (1998). *J. Am. Chem. Soc.* **120**, 3265–3266.