Gas-phase spectroscopy of a protein

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Abstract. We present preliminary results on gas phase photoionization of electrospray-produced multiply protonated cytochrome c protein (104 amino acids; ≈12.4 kDa), which has been achieved with an experimental system for spectroscopy of electrosprayed ions in the ion trap using synchrotron radiation. The present results are the first reported on photoionization of kDa species in the gas phase and are valuable regarding fundamental interest of accessing physical properties of large biological species isolated in vacuo.

1. Introduction

The investigation of proteins in the gas phase, where they are free of the influence of counterions and solvent molecules, offers a possibility to understand their intrinsic molecular properties. However, due to limited both ion density and available photons flux, the use of synchrotron radiation for the trapped ions spectroscopy is a rather challenging task. The feasibility of coupling a Fourier transform ion cyclotron resonance ion trap with soft x-ray synchrotron beamline and the first successful use of synchrotron radiation for spectroscopy of electrosprayed negative ions stored in a three-dimensional quadrupole ion trap have been demonstrated only recently [1,2].

The possible efficiency for the ionization of large molecules has attracted considerable discussion some years ago. Shlag et al (1992) argued that ionization efficiency strongly diminished with increasing the mass of the molecule suggesting that “a very large molecule can act at its own solvent”. They have suggested that in the large molecules, the ionization occurs through the intermediate formation of charge pairs which can ionize in a unimolecular-like fashion; nevertheless, a more probable route was considered for the charge pair to recombine. Shlag et al [3] also presented the experimental observation that the ionization efficiency rapidly decreases with increasing the weight of the molecule, whether one uses electron impact, single-photon or multi-photon ionization. The least efficient process was found for the single-photon ionization. It should be noted, however, that the experiment used only 10.5 eV photons (118 nm generated by frequency tripling the third harmonic of
a Nd:YAG laser), while the cross sections were not discussed. Later theoretical work by Thoss and Domcke [4] on a model study of nearthreshold PI of large molecules indicated that, indeed, vibrational relaxation may be an important mechanism suppressing the ionization process. A more profound discussion and a review on the issue of the ionization efficiency of large molecules can be found in a later paper by Berkowitz [5]. The author concludes that the ionization of large molecules should be possible but indeed with increased difficulty. Note, finally, that removing an electron from a positive ion, which is the present case, is expected to be even more difficult due to the attractive Coulomb field.

According to our knowledge, the first experimental evidence that irradiation of large polyatomic ions by electrons at energies above the ionization threshold causes the ionization of the latter species has been presented by Zubarev group at the beginning of 2000s [6,7]. The authors have measured the ionization energies, obtained by tandem ionization in Fourier transform mass spectrometers, for different polyprotonated peptides (up to about 3.5 kDa) and different charge states of the precursor ions. They reported a dependence of the ionization energies of protonated polypeptides on the number of charges, where a clear trend of the increase in the ionization energy with the charge was found, the likely reason for which is the Coulombic effect. When put on a plot, the IE data strongly suggested a linear dependence. Most recently, the same group reported electron ionization of multiply charged ubiquitin protein (≈8.6 kDa) cation, in the frame of promoting a new MS2 technique – electron ionization dissociation (EID) [8]. The later paper by Fung et al [8] neither discusses the ionization efficiency curves nor the threshold for the electron ionization of ubiquitin. Finally, it should be noted that Kalcic et al [9] has recently reported femtosecond laser-induced ionization/dissociation of protonated peptides (angiotensin II and GAILpTGAILK – up to about 1 kDa), obtained by coupling an Ti:Al2O3 laser with a modified Thermo Scientific LCQ DECA XP Plus ion trap mass spectrometer. This photoionization process involves the use of ultrashort (<35 fsec) laser pulses of 28 nm bandwidth centered around 800 nm, resulting in a nonergodic dissociation.

In the present paper, we report on the experimental evidence of a single photon ionization of a large polyprotonated biopolymer in the gas phase. The measurements were performed by means of coupling a commercial linear ion trap with a VUV synchrotron beam line. A special care has been taken to design a system which would allow a perfect alignment and large overlap between the trapped ion volume and the photon beam, which resulted in an increased sensitivity of the experiment and large signal to noise ratio.

2. Experimental setup
The experimental setup for spectroscopy of electrosprayed ions was coupled with the DESIRS beamline at the SOLEIL synchrotron radiation facility (Fig. 1). The DESIRS undulator emission allows measurement in the 4-40 eV photon energy range, although for the present study we only used the 4-20 eV range. The wavelength is selected by a normal incidence monochromator, using a grating of 200 gr mm\(^{-1}\) which provides a high photon flux \((10^{12} – 10^{13} \) photons/s) with a photon resolution of typically 12 meV at 10 eV photon energy with a 200 µm exit slit. The photon beam can be filtered out for high harmonics using a gas filter.

The experimental system was based upon a commercial linear quadrupole ion trap (“Thermo scientific LTQ XL”), equipped with the ESI probe. The electrosprayed ions are introduced from the front side of the spectrometer and guided through the system of multipole ion lenses into a two-dimensional quadrupole ion trap [10]. The trapping region is formed as a cylindrical volume, which allows improved overlap with the synchrotron beam with respect to Paul ion traps. The synchrotron beam is introduced into the trap through the back lens of the spectrometer. A special frame has been constructed to allow fine-tuning of the position of the spectrometer, i.e. of the trapping region, with respect to the light beam. The mechanical frame is made of five different plates, so the mass spectrometer that is mounted on the top of the system can be positioned with respect to four independent movements: direction along the beam, 2 directions normal to the beam and rotation of the cylindrical trapping region in the horizontal plane.
Figure 1: Coupling of the DESIRS beamline at SOLEIL synchrotron facility with the LTQ XL mass spectrometer (Thermo Scientific).

The vacuum manifold with a turbo pumping stage has been designed to accommodate pressure difference between the beamline (10^{-8} mbar) and LTQ (10^{-5} mbar). A turbo pump of 300 L/s was used for the present purpose. The manifold also includes a suprasil window for additional filtering of high order radiation at lower photon energies. A photodiode for the photon flux measurements and the photon beam shutter are placed inside a small cube, which is connected to the back plate of the LTQ mass spectrometer through a flexible bellow allowing the alignment. A new design of the shutter, with an electro-motor (from the brand “KUHNKE”) inside the vacuum attached to a copper heat-sink, allowed achieving short (about 1 ms) and reproducible chopping time under high-vacuum conditions, with a good reliability during all the experimental time.

An acquisition system was made to synchronize the beamline energy scanning with the trapped ions activation, thus allowing an efficient collection of large amount of data (a number of tandem mass spectra averaged over desired time interval for a desired photon energy). A home-made PC application synchronizes the ESI injection, mass spectra recording and the beam line photon energy change. Multiply protonated cytochrome c molecules are generated in the ESI source from water/ACN (75:25) solution (with 0.2% of pure ammonia) at 10 µM bovine cytochrome c (Sigma Aldrich).

The geometry of the linear quadrupole ion trap and the experimental procedure is shown in Fig. 2. The sequence of events is as follows. Electrosprayed ions are injected, selected and stored in the trap; when the desired ion capacity is reached, the beam shutter opens, thus starting the irradiation; after desired time of irradiation, the shutter intercept the beam; the mass spectrum is recorded; the monochromator is set to the next wavelength and the procedure is repeated. The recorded mass spectra can be processed during the measurements or afterwards.
Figure 2: Schematic drawing of the experimental procedure.
3. Results
The ESI/photon-activation tandem mass spectrum recorded after about 80 ms irradiation of the
[M+4H]⁴⁺ isolated protonated protein ions, at the photon energy of 15.5 eV, is shown in Fig. 3. The
dominant peak at about m/z=3080 corresponds to the precursor ion, selected from the ESI spectrum.
Nevertheless, an additional feature peaking at about m/z=2460 that corresponds to an odd electron
radical product cation [M+4H]●⁵⁺, formed via photoionization of the [M+4H]⁴⁺ precursor, is clearly
seen.

![Figure 3: ESI/photon-activation tandem mass spectrum of cytochrome c protein obtained with 80 ms
of irradiation time, at the photon energy of 15.5 eV.](image)

The presented result shows a very efficient photoionization of the polyprotonated cytochrome c
protein upon VUV radiation. The measurements of the ionization efficiency as a function of the
irradiation time (in the range from about 50 ms up to 400 ms) showed a linear dependence, thus
confirming a single photon process. Further work will be focused on the photoionization efficiency
measured as a function of the photon energy, which will allow to determine ionization thresholds for
different protein charge states. The results will be valuable to ascertain fundamental physical
properties of the polyprotonated biopolymers isolated in vacuo.
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§ http://www.synchrotron-soleil.fr/Recherche/LignesLumiere/DESIRS