HIGHLY CHARGED IONS COLLISIONS WITH MULTIPLY CHARGED PROTEIN ANIONS STORED IN A LINEAR ION TRAP

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Abstract. We have investigated 375 keV Xe^{25+} ions collisions with cytochrome *c* (≈12.5 kDa) and BPTI (≈6.5 kDa) gas-phase protein anions of selected charge states. The experiment has been performed by coupling a linear quadrupole ion trap mass spectrometer, equipped with the electrosprayed ions probe, to the highly charged ions (HCIs) beamline. We report the first results on HCI interaction with stored protein anions, showing a multiple electron capture accompanied by neutral losses, as dominant relaxation channels.

1. INTRODUCTION

There is a large interest to study high-energy particles (electrons, photons, ions) collisions with biopolymers (proteins and DNA) isolated and maintained under well-defined conditions in the gas phase. Such studies can improve our understanding of the basic physicochemical properties of biomolecules, as well as the interplay between these properties and their function [1]; lead to important new applications such as novel methods for protein sequencing [2]; and support research on radiation damage and novel medical methods such as ion-beam cancer therapy [3]. However, it is experimentally very challenging to bring large biomolecules such as proteins and DNA intact into the gas phase and perform standard collisional experiments under well-defined conditions.

We have recently developed an experimental setup [4] that couples a linear ion trap with the synchrotron beam, thus allowing to perform controlled

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investigation on energetic photon (VUV and X-ray) interaction with large multiply charged biopolymers and to study, for the first time structure and charge-state dependence of the gas-phase VUV ionization of proteins [1] and X-ray inner-shell excitation/ionization of proteins [5].

Most recently, we have extended our study to collision with ions, in order to investigate highly charged ions interactions with multiply charged protein ions stored in a linear ions trap. Particularly, we have studied the interaction of 375 keV Xe^{25+} ions with cytochrome *c* (≈12.5 kDa) and BPTI (≈6.5 kDa) gas-phase protein ions of selected charge states as a function of the precursor charge state.

2. EXPERIMENTAL SETUP

The experimental setup is based on a commercial linear quadrupole ion trap ("Thermo scientific LTQ XL"), equipped with an electrospray ion source (ESI), which has been coupled to the ARIBE beamline, the low-energy ion beam facility of the GANIL in Caen, France [9]. A detailed description of the experimental setup, which has been previously coupled to synchrotron beamlines, is given elsewhere [4,6]. Briefly, the target ions were produced by ESI and introduced from the front side into the ion trap; after isolation of the desired precursor, the Xe^{25+} ion beam was introduced into the trap through the back lens of the LTQ XL spectrometer. 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).

Multiply deprotonated cytochrome *c* and BPTI (Sigma Aldrich) molecules were generated by the ESI source from water/acetonitrile (75 : 25) solution at 10 mM. Negative protein precursors have been isolated in the gas phase in the ion trap and submitted to 375 keV Xe^{25+}. The mass spectra have been repeatedly recorded for a specific precursor, after an irradiation time of about 1 s. For each charge state, the background signal (both without the incident ion beam and without the targets ions in the trap) has been recorded, as well.

3. RESULTS AND DISCUSSION

Cytochrome *c* has been investigated previously in our work on X-ray interaction with trapped biopolymers [5] and very recently in collisions of 96 keV Xe^{8+} ions [7]. The present contribution present results for a selected -11 charge state precursor. BPTI protein has been investigated further, representing a complex biopolymer, which should not unfold in the gas phase (until some degree of protonation) due to disulfide bridges [1].

Figure 1 shows tandem mass spectra upon Xe^{25+} ion activation of deprotonated cytochrome *c* (a) and BPTI (b) proteins. Except the dominant peak corresponding to the precursor ion, additional peaks at higher m/z, which correspond to the electron capture by the highly positively charged Xe projectile, are clearly seen. The electron capture, i.e. electron detachment from the precursor protein ion to one Xe^{25+} orbital, is accompanied by intensive neutral

losses. In both cases, the dominant loss corresponds to the mass of about 44 Da, thus suggesting a possible loss of CO_2; however, neutral losses from the amino acids cannot be excluded, therefore more detailed measurements with higher mass resolution are needed in order to perform an accurate analysis. Still, it is interesting to note that the dominant reaction channels upon highly charged ion collisions with trapped proteins anions represent the electron detachment (captured by the incident projectile) accompanied by neutral losses, whereas backbone fragmentation is less abundant. This might be regarded as a surprise considering previous results with amino acids [10] and peptides [8], however, this is actually in line with the our most recent study on soft X-ray [5]. Indeed, the susceptibility of proteins biopolymers to energetic ion bombardment is significantly different in comparison with their monomer units (amino acids), suggesting that a so-called building blocks approach may not be relevant in this particular case.

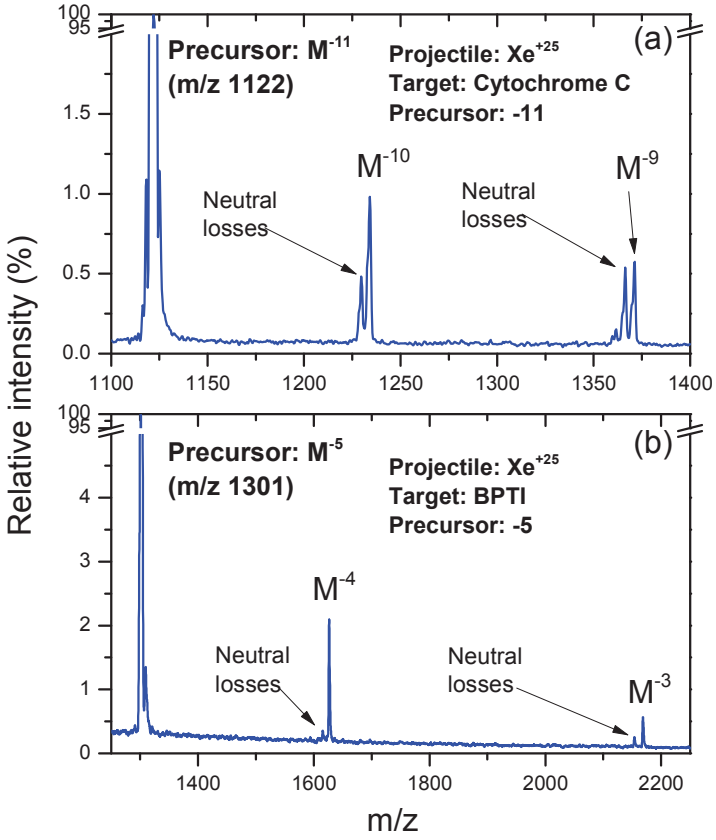

Figure 1. Tandem mass spectra of -11 charge state precursor of cytochrome *c* protein (a) and -5 charge state precursor of BPTI protein (b) upon activation by 375 keV Xe^{+25} projectiles.

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