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Femtosecond laser induced ionization and dissociation of gas-phase protonated leucine enkephalin

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ABSTRACT

We have combined a tandem mass spectrometer with a 780 nm fs-laser system to study photoionization and photofragmentation of trapped protonated leucine enkephalin cations for laser intensities between 2×10^{13} W/cm^2 and 1×10^{14} W/cm^2 and pulse durations of 15 fs. In this intensity range, the transition from multiphoton ionization and excitation to tunneling ionization is expected to occur. The observed partial ion yield curves as a function of laser intensity exhibit a power-law dependence, indicating multiphoton absorption to be the dominating mechanism. Pump-probe studies were performed to investigate the time-evolution of the multiphoton ionization process. The partial ion yields of almost all fragmentation channels show a broad but distinct maximum at a delay-time of approximately 750 fs. The particularly flat appearance of the pump-probe curves suggests that not a single resonance, but a broad distribution of resonances is involved.

1. Introduction

The combination of electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI) with tandem mass spectrometry has developed into a standard tool for peptide and protein structure analysis [1]. Typical instruments rely on molecular excitation, for instance by multiple collisions with inert gas atoms (collision-induced dissociation, CID [2]) or by collisions with surfaces (surface induced dissociation, SID [2]), by blackbody infrared absorption [3,4] or by absorption of photons from UV lasers [5]. Furthermore, complementary charge-changing techniques such as electron capture dissociation (ECD) [6–8]) or absorption of VUV photons [9,10] and soft X-rays [11,12] can be employed.

A relatively new development is the combination of high power femtosecond laser pulses with mass spectrometry techniques. Depending on the laser peak electric field, ionization/excitation can be explained either due to multiphoton absorption or electron tunneling, leading to the efficient production of product ions that are often not accessible by conventional techniques.

In pioneering experiments, Kalcic et al. [13] have interfaced a high-power fs-laser with a commercial ion-trap mass spectrometer to compare dissociation of protonated peptides by CID and by fs laser induced ionization/dissociation (fs-LID) using intense pulses (10^{13}–10^{14} W/cm^2) of 33 fs duration. For fs-LID of singly protonated peptides, a fragmentation pattern of much better sequence coverage was obtained, compared to what is achievable with CID. Therefore, it was concluded that the technique allows for sequence analysis of singly protonated peptides. In a subsequent study on protonated phosphopeptides, it was shown that fs-LID is ideally suited to suppress the loss of labile neutral groups and to prevent phosphate group rearrangement prior to dissociation [14]. Phosphorylation can thus be unambiguously characterized.

In a similar type of experiment, Duffy et al. [15] have studied the interaction of 100 fs pulses at 800 nm and at 267 nm for intensities in the $\approx 10^{11}$–10^{13} W/cm^2 range with laser desorbed neutral amino acids and peptides. Mass spectra dominated by immonium ions were observed, which was explained by ionization of the most weakly bound molecular orbitals from either the amino group

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N lone pair or from a sidechain. Interestingly, at 800 nm the N-terminal immonium ion yields for protonated peptides are higher than those for the respective isolated amino acids, suggesting photoionization induced charge migration.

Surprisingly, the ultrashort nature of the pulse itself has not been directly exploited, yet, in pump-probe type experiments. Clearly, fs pulse durations are predestined for time-resolved studies of molecular dynamics on fs timescales.

In this article we thoroughly investigate the fs-LID as a function of laser intensity for the protonated peptide leucine enkephalin ($[YGGFL+H]^+$ with the amino acid residues glycine (G) and leucine (L) (both aliphatic) as well as tyrosine (Y) and phenylalanine (F) (both aromatic). Laser intensities are varied from 10^{13} to 10^{14} W/cm^2. In this region the transition from multiphoton ionization to tunnel ionization is expected to take place. Subsequently, a dual-pulse pump-probe scheme is employed to investigate the peptide dissociation dynamics in strong laser fields with fs time resolution.

2. Experiment

The experimental data have been obtained by interfacing a tandem mass spectrometer [16], home-built at the University of Groningen with the fs-laser system at the Max-Planck-Institut für Kernphysik. The experimental setup is sketched in Fig. 1.

2.1. Mass spectrometer

Briefly, singly protonated $[YGGFL+H]^+$ cations were produced in an electrospray ionization source. The peptide was dissolved in methanol solution with a concentration of 36 μM and with addition of 1% of formic acid.

Electrosprayed ions were collected using a radiofrequency (RF) ion funnel and guided into a collisionally focusing RF-only quadrupole for phase-space compression. An RF-quadrupole mass analyzer was used to select the desired $[YGGFL+H]^+$ ions. The protonated peptides entered a three dimensional RF-quadrupole ion trap through one of its end-caps. The base pressure inside the trap chamber was 1×10^{-9} mbar. Trapping of the $[YGGFL+H]^+$ ions was achieved by means of collisional cooling with a helium buffer gas, which was applied during the trap loading phase using a solenoid valve. Typical buffer-gas pressures inside the trap were estimated as about 1×10^{-3} mbar and typical trap loading periods were a few 100 ms. Outside the loading period, the peptide ion beam was electrostatically deflected. A period of about 100 ms, leading to a pressure drop into the low 10^{-5} mbar range, was found adequate to allow for almost background free investigation of the photoionization process. The RF ion trap was operated at ≈1 MHz at peak-to-peak voltages around 2 kV. Under these conditions, only ions with an m/z exceeding ≈70 were trapped, which is why the relatively high background pressure did not affect the experiment.

The trap content was then exposed to laser pulses at a wavelength of 780 nm. The estimated pulselength was ≈12 fs, at a repetition rate of 3 kHz with powers between 30 mW and 300 mW. Depending on the laser power, the trap content was exposed to 150–600 pulses (50–200 ms). Under these conditions, typically much less than 10% of the trapped protonated peptides were photoionized and/or photodissociated, i.e. less than 10% of photoionized peptides interacted with more than one independent laser pulse. The actual contribution of double-pulse ionization to the observed mass spectra is expected to be even lower, as such processes involve photodissociation of photodissociation products. Mostly very small fragments were created with an m/z below the trap threshold.

After the laser pulses, a second He-buffer-gas pulse was applied to the trap, to collisionally cool dissociation products with high kinetic energies. Trapped protonated peptides and photoionization products were then extracted into a linear time-of-flight (TOF) mass spectrometer ($M/\Delta M \approx 250$) by applying a bias voltage ($U_{bias} \approx \pm 200$ V, duration: 5 μs) to the RF-trap endcaps. The ions were detected by a micro-channel-plate detector and read out by means of a 1 GHz digitizer. Typically, averaging of approximately 1000 digitizer traces yielded sufficient statistics for a good quality mass spectrum.

To reduce the influence of laser interactions with residual gas molecules and to account for fluctuations in the peptide flux from the ESI source, data were acquired in 3 successive sub-scans through which the experiment cycled repeatedly: (i) an "inclusive" TOF spectrum according to the description above; (ii) a TOF spectrum of electrosprayed peptides *without* photoabsorption (laser off); (iii) a TOF spectrum of the photoionized residual gas (ESI off). The latter two spectra were then subtracted from the inclusive scan. A three-scan cycle took about 2–3 s.

2.2. Laser system

The laser pulses were delivered by the femtosecond Ti:Sa laser system installed at the Max-Planck-Institut für Kernphysik in Heidelberg [17], which features a repetition rate of 3 kHz, a pulse length of 25 fs and a pulse energy of 1 mJ.

A subsequent fiber compressor was used to further reduce the pulse length to about 12–15 fs. To achieve this, the pulse bandwidth is broadened by passing the beam through a 90 cm Ne-filled hollow glass fiber with an inner diameter of 250 μm. This configuration ensures high laser intensity throughout the fiber with the Ne acting as nonlinear medium. A mirror compressor consisting of 6 chirped mirrors is used to compress the pulses to the desired length, taking into account the positive dispersion of air and glass on the way to the interaction region.

For the optional pump-probe mode, the laser beam can be passed through a Mach–Zehnder interferometer. Here, a beam splitter is used to separate 50% reflected and 50% transmitted light. In one interferometer arm, two mirrors are mounted on a translation stage. This allows precise variation of the difference in length of the interferometer arms (see Fig. 1). A second beam splitter is then used to overlap the two beams in a way that both beams undergo transmission and reflection, once. Since both beam splitters are identically constructed, both pulses undergo the same amount of dispersion resulting in two identical pulses with variable time separation.

The laser beam is focused into the center of the RF-trap by means of a focusing mirror with a focal length of 0.4 m resulting in a focus diameter of about 40 μm. Laser intensities correspondingly range from 10^{13} to 10^{14} W/cm^2.

3. Results and discussion

3.1. Mass spectra

Typical mass spectra for multiphoton ionization of protonated leucine enkephalin $[YGGFL+H]^+$ are displayed in Fig. 2 for the intensities 2.8×10^{13} W/cm^2 (top), 5.5×10^{13} W/cm^2 (center) and 1.1×10^{14} W/cm^2 (bottom). The low mass cutoff of the RF-ion trap is around $m/z = 70$. Fragments with m/z exceeding 318 are only formed with negligible intensities and accordingly, only the m/z range from 70 to 350 is displayed. Clearly, the partial ion yields, i.e. the peak intensities, exhibit the expected global increase with laser intensity (note the different intensity scales in Fig. 2). Clearly, the spectrum obtained at 2.8×10^{13} W/cm^2 is dominated

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