LIAD-fs: A novel method for studies of ultrafast processes in gas phase neutral biomolecules

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Abstract. A new experimental technique for femtosecond (fs) pulse studies of gas phase biomolecules is reported. Using Laser-Induced Acoustic Desorption (LIAD) to produce a plume of neutral molecules, a time-delayed fs pulse is employed for ionisation/fragmentation, with subsequent products extracted and mass analysed electrostatically. By varying critical laser pulse parameters, this technique can be used to implement control over molecular fragmentation for a range of small biomolecules, with specific studies of amino acids demonstrated.

1. Introduction

With recent developments in ultrashort light sources, the time- and space-domain of molecular dynamics can now be approached with unprecedented resolution. Whilst fundamental molecules are ideal for proof of principle experiments and for elucidating fundamental dynamics (e.g. see [1] and references therein), there are now significant rewards to be reaped by studying more complex systems, such as those of biological interest. For example, in the space domain, the recently commissioned LCLS facility provides coherent X-ray pulses capable of diffractive imaging of single biomolecules [2].

With regard to the time domain, one promising avenue of research is in the application of intense femtosecond (fs) laser technology to biological molecules. Femtosecond lasers can provide interactions on timescales shorter than structural rearrangement and charge transfer processes, and supply strong electric fields which are capable of non-resonantly distorting potential landscapes and directing the outcome of molecular fragmentation events. Significant promise has already been demonstrated in fs sequencing of peptides [3] and of selective bond cleavage in prototypal amino acids using shaped pulses [4]. In this article, we introduce a new technique for studying fs interactions with biomolecules, which provides a platform for future time-resolved studies of ultrafast processes in these systems.

2. Experimental Technique

2.1. Laser Induced Acoustic Desorption

In order to study fragmentation dynamics for fs laser interactions with individual molecules, the ability to produce a gas phase target is paramount. For studies of biomolecules in the gas phase, the pioneering methods of electrospray ionisation (ESI) [5] and matrix assisted laser desorption/ionisation (MALDI)[6] are widely used. These soft ionisation techniques enable
Figure 1. Schematic of LIAD-fs in the KEIRAlite ion trap. A biomolecular sample is deposited on a Ta foil (a) which is secured to sample electrode 1 in KEIRAlite. The foil is then back-irradiated using a UV ns pulse (b) and a femtosecond laser (c) is interacted with the resulting biomolecular plume. Charged products are extracted and analysed electrostatically (d) using the electrodes in KEIRAlite. Products are detected either via direct time-of-flight (ToF) or ion trapping. For ToF measurements, the reflection electrodes are earthed and products are detected at a CEM detector. For ion trapping, the reflection and sample electrodes are used to create an electrostatic cavity in which the ions are stored on stable trajectories and ion detection is implemented using pickups P1-P3 as image-charge detectors.

studies of thermally labile, non-volatile molecules, where ionisation typically occurs via proton attachment/detachment. Whilst these methods have provided significant progress in studies of biomolecular photoabsorption and fragmentation, there are limitations to their scope. In ESI, a gas phase sample is created using polar molecules that easily undergo ion (or proton) attachment (or detachment) and is therefore often a modified form of the biomolecule of interest. ESI is also known to produce low number densities, such that a multipole ion trap is generally required to accumulate ions prior to any photon induced studies. In MALDI the preparation conditions must be tailored to the desired sample (analyte), with the resulting gas phase target containing matrix molecules and associated solvents. Therefore any mass spectrum arising from an intense fs interaction with this target will be polluted by these contaminants.

As an alternative method for preparing our gas-phase target, we use Laser Induced Acoustic Desorption (LIAD) [7]. In contrast to the above techniques LIAD enables us to produce a pure, neutral target of biomolecules and can be applied as a general technique for a wide range of molecules. The ability to introduce significant quantities of intact neutral biomolecules into the
gas phase in this way, is ideal for conducting fundamental investigations of conformational and structural dynamics of amino acids, polypeptides, and DNA bases [8].

The LIAD-fs technique is depicted in Figure 1, where a biomolecular sample (a) is deposited on the surface of a thin Ta foil which is then back-irradiated, using a nanosecond (ns) UV pulse (b). This interaction stimulates the propagation of an acoustic wave through the foil, resulting in sample desorption from the surface. After a chosen delay time an fs pulse (c) interacts with the desorbed neutral target, with charged ionisation/fragmentation products extracted and mass analysed electrostatically (d) using our KEIRAlite ion trap mass spectrometer, detailed in Section 2.2.

The biomolecular samples were prepared as follows. The Ta foil (Goodfellow), of 10 µm thickness and 8 mm diameter, was secured to a sample plate, using epoxy-resin vacuum compatible glue. Molecular samples (Sigma-Aldrich) were typically prepared in 0.01 - 0.05 molar aqueous solution. This solution was deposited on the foil in several successive applications, using a vacuum drying station at each stage, with 10 - 20 µl deposited in total. For each new sample, a new foil was prepared.

The UV laser pulses used to back-irradiate the foil were produced by an Opotek Opolette laser, providing 355 nm pulses of ~4 ns duration and ~0.3 mJ which were focused onto the foil to provide pulse intensities in the range 10^8 - 10^9 W/cm^2. The ns UV laser was synchronised to the fs system, a Coherent Libra regenerative amplifier providing 1 mJ pulses at λ = 800 nm and with pulse duration of 100 fs. Both laser systems were operated at 20 Hz, with the UV-fs delay (between the Opotek and Libra systems) typically set in the range 10 - 30 µs with the fs laser focus typically positioned at ~1 mm from the surface of the sample plate.

2.2. KEIRAlite: Ion Trap Mass Spectrometer

Ionisation/fragmentation products from the fs-biomolecule interaction were detected in KEIRAlite, a linear electrostatic storage device [9] in which ion trajectories can be axially confined between two electrostatic mirrors. It is an advanced form of its predecessor KEIRA (Kilovolt Electrostatic Ion Reflection Analyser) [10], with a more compact design (both in the size and number of electrodes) and integration of a LIAD biomolecular ion source. The electrodes are cylindrically symmetric (outer diameter 46 mm) and the full trap is mounted on ceramic rods and housed in a compact ConFlat vacuum chamber (DN63).

The LIAD sample plate was inserted into electrode 1 of the KEIRAlite apparatus which was then evacuated using two turbo molecular drag pumps backed by a scroll vacuum pump, down to an operating pressure of < 10^-7 mbar. Ions were created via the LIAD-fs technique between sample electrodes 1 and 2 which were typically maintained at 5 and 4.7 kV respectively, such that positive ions were extracted along the central trap axis. Mounted between electrodes 2 and 3 is a resistive glass cylinder (Photonis) which provides a uniform electric field between these two electrodes (with the latter typically held at 0 kV). This means that ions passing through the region along the central axis of the trap see a smoothly decreasing potential surface. Electrode 4 is used as the central element of an einzel lens, to provide radial confinement of ion motion. If identical voltages are applied to electrodes 1 - 4 on the reflection end, the ions can be stored in the trap, oscillating back and forth on stable trajectories. Alternatively by earthing the reflection electrodes, the ions can be transmitted to a channel electron multiplier (CEM) where time-of-flight (ToF) measurements enable conversion to a mass spectrum. In this article, mass spectra obtained via ToF measurements are displayed, where the data collection was triggered using a photodiode to detect the fs pulse, and typically averaged over 1000 LIAD-fs events.

3. Results and Discussion

Results from a LIAD-fs study of the amino acid histidine are shown in Figure 2, with the structure of histidine given in the inset (C6H9N3O2, mass = 155 u). For the results displayed
in Figure 2(a), the UV-fs delay was set at 20 μs and the fs pulse was focused onto the neutral plume with a peak intensity of \( \sim 5 \times 10^{14} \) W/cm\(^2\). It is immediately apparent that the majority of ion signal is detected in the mass range 10 - 50 u, such that the neutral histidine target is being substantially fragmented.

Significantly less fragmentation is seen in Figure 2(b), where a cylindrical lens (20 cm focal length) was inserted in the fs beam path, to increase the interaction volume at lower peak intensity. The histidine parent ion (M\(^+\)) is observed at 155 u, and the peak at 110 u is due to the loss of the carboxyl (COOH) group from the parent ion (breakage of bond 2). The sidechain (R\(^+\)) of the amino acid (breakage of bond 1) is observed at 81 - 82 u, and is the most dominant peak in the spectrum. The measured yield for the fragment (M-R)\(^+\), peak 3, is much smaller than that of R\(^+\) indicating that the charge is much more likely to reside on the sidechain upon fragmentation. This is especially prevalent in the lower intensity plot (b).

From this data, it is clear that the fs interaction enables significant fragments to be observed that can be attributed to the histidine molecule, with the observation of the sidechain of the amino acid. Moreover, the data in Figure 2 clearly demonstrates that manipulation of the laser intensity provides a means to control the degree of molecular fragmentation.

Results are displayed in Figure 3 for a LIAD-fs study of another amino acid, lysine. These were acquired at a UV-fs delay of 20 μs and fs intensity of \( \sim 10^{13} \) W/cm\(^2\). Lysine is an α-amino acid of mass 146 u and sidechain, \((\text{CH}_2)_4\text{NH}_2\), of mass 72 u. Similar to the histidine studies, we found the pulse intensity to play an important role in the degree of fragmentation of the molecule, with the data shown here acquired using the cylindrical lens to provide the low intensity interaction. In this data, the parent ion (M\(^+\)) is clearly seen at 146 u, as is the sidechain (peak 5).

Indeed in the mass spectrum, there is evidence for breakage of each bond along the sidechain. For ease of reference, we have labelled each bond along the sidechain of the structure from 1 to 5, and annotated the mass peaks accordingly. For each peak, the colour of the number denotes the side of the molecule that is observed, i.e. where the charge resides after fragmentation. If the charge resides on the sidechain fragment the number is purple, and if the charge resides on the amino acid backbone, the number is green.

For example, the breakage of bond 5 could result in two different fragments, the sidechain at mass of 72 u (purple 5), and the amino acid backbone at 74 u (green 5). By comparing these
peaks, the mass spectrum indicates that the charge is more likely to remain on the sidechain, as the peak 5 at 72 u is much larger than that at 74 u.

Using this notation, it appears that all bonds along the sidechain (bonds 1 - 5) can be broken by the fs laser interaction, with the fragments clearly observed. It is worth noting that the Cα-C bond on the amino acid backbone may also be broken (as was seen with ‘bond 2’ in histidine), denoted by bond 6 here. This breakage could correspond to fragments at 101 u (M-COOH)⁺ and 45 u (COOH)⁺, coinciding to similar masses as the breakage of bond 3 which would give (C₂H₄NH₂)⁺ at 44 u and (M-C₂H₄NH₂)⁺ at 102 u. Whilst there is some evidence of a small peak at 44 u, the peak at 101 u is most likely to be (M-COOH)⁺ fragment. Similar to the histidine case (where (M-COOH)⁺ was seen at 110 u), for this breakage there is no contribution from (COOH)⁺ but rather the charge resides on the (M-COOH)⁺ fragment.

With the range of assignable peaks observed in Figure 3, this data provides a clear ‘fingerprint’ for the lysine molecule, with its fragmentation pattern well characterised for these LIAD-fs conditions. In contrast to this, it worth noting that electron ionisation mass spectra of lysine [12] do not display any of the masses > 100 u, that are seen so clearly here in the LIAD-fs technique.

In a separate experiment (not shown), a sample was prepared containing both lysine and histidine, and LIAD-fs experiments carried out. The resulting mass spectrum was consistent with a linear combination of the separate fingerprints from Figure 2(b) and Figure 3. This is of interest for mass spectrometry, where comparison of an unknown sample with a library of known samples could enable identification of constituent parts.

4. Conclusions
The results reported here demonstrate the capability of the LIAD-fs technique for gas-phase studies of biomolecules. By controlling the fs pulse intensity, we have established that fragmentation can be significantly suppressed.

To date, LIAD has been most effectively exploited in mass spectrometry studies, in conjunction with a secondary ion activation scheme; such as chemical ionisation (CI) [13], electron ionisation [14] or single photon ionisation [15]. Of these methods, CI is the preferred choice in the literature and is regarded as the most efficient ionisation method which can be ‘tuned to favour particular chemical components’ (see [16] and references therein). In this context we note that femtosecond laser pulses also provide highly efficient ionisation, have the potential to be tuned to alter fragmentation patterns via manipulation of basic pulse parameters (pulse duration/intensity) or indeed via spectral pulse shaping [4]. Not only does the LIAD-fs
approach outlined here provide a useful scheme for ultrafast studies of biomolecules, but it also has significant potential in mass spectrometry.

In terms of future studies of ultrafast dynamics, these formative studies have been carried out using pulses of 100 fs duration, and give insight into how these small biomolecules behave in an intense laser field. This provides a valuable platform for future experiments where ultrashort pulses (< 10 fs) promise to enable observation and control of biomolecular fragmentation on sub-vibrational timescales, and investigate ultrafast dynamics such as vibrational wavepacket evolution and charge migration [17].

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