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Abstract. Fragmentation of doubly charged biomolecules, uracil and amino acids, has been investigated using different ab inito Molecular Dynamics Methods. Time-Dependent Density Functional Theory Molecular Dynamics give a description of the non-adiabatic effects, the charge redistributions that occur in the first few femtoseconds and reveal the importance of the chemical environment. The combination of different techniques allow us to interpret the complex multicoincident spectra obtained experimentally when the molecules collides with ions or are excited with synchrotron radiation.

1. Introduction

The interaction of biomolecules with ionizing radiation, either high-frequency electromagnetic radiation or energetic ions, is a topic of primary importance to understand the underlying physical mechanism of radiation damage and modern cancer treatments based on the use of swift ions as H^+ or C^{6+} (hadron-therapy)[1] where energy is deposited in a very localized area (Bragg peak)[2]. Cell damage can be produced by direct excitation or ionization of the DNA[3,4], or indirectly, by chemical attack of the radicals, formed in the surrounding water environment, to the DNA [5]. Although single ionization is the most probable and best-studied mechanism; swift ions can also induce multiple ionization events along their tracks (around 10% of the primary ionization ones)[6]. Previous studies

suggest that inner shell ionization of DNA constituent atoms, leading to double ionization events after Auger effect, could be partly responsible for cellular inactivation upon heavy ion[7] and γ-ray irradiation[8]. These events were shown to induce irreparable damage in cellular DNA when produced in conjunction with low energy electrons[9]. Therefore the stability of doubly and multiply charged species is a topic of large importance to fully understand radiation damage. Fragmentation is the main mechanism to relax the excess of energy associated with the creation of vacancies in electronic shells of the molecules. For multiply charged species fragmentation in several charged fragments (process known as Coulomb explosion) also relaxes the instability induced by the presence of multiple localized charges in the same molecule. The fragmentations of highly charged and excited molecules usually occurs in a very short time scale, of the order of femtoseconds, although delayed fragmentation of multiply charged species has been also reported [10-12]. The very early stages of the fast fragmentation dynamics are far from being well understood.

Recent experiments in the gas phase allow to study single-molecule fragmentation events of doubly charged species using multicoincident detection techniques, that permit to correlate all the ions produced after ionization[13-16]. Double ionization can be induced in collisions with ions or by using synchrotron radiation. In these latter experiments electrons can be extracted from different inner shell orbitals. Further decay from these core-hole states produces the emission of a second electron due to the Auger effect, leaving the molecule in a doubly charged and excited state. Photoelectrons, Auger electrons and charged fragments can be measured in coincidence. Kinetic energies of the emitted fragments can also be measured, helping to unravel the fast mechanism involved in Coulomb fragmentations.

The study of the fragmentation of doubly charged and excited biomolecules represents a challenge from the theoretical point of view; electron relaxation and nuclear fragmentation dynamics occur in a time scale of femtoseconds and both electrons and nuclei dynamics should be treated simultaneously and cannot be captured within the Born-Oppenheimer approximation. Simulations are crucial to understand how fragmentation patterns depend on the shape of the molecular orbital from which the electrons are extracted. They can also help to highlight the role of the intramolecular environment and to elucidate the structure of the fragments produced. Also comparison of simulations performed in the presence/absence of surrounding water molecules can give new insights on the role of the solvent.

The aim of the present article is to review different theoretical approaches that can be used to understand the stability of doubly charged biomolecules and to follow their fragmentation dynamics (section 2). We will also review some recent results obtained for different biomolecules, in particular in uracil^{+2} both in gas-phase and aqueous solution to illustrate how the chemical environment affects the fragmentation dynamics (section 3) and in doubly charged amino acids to show how different processes compete during fragmentation (section 4). Finally (section 5) we will summarize the most important conclusions.

2. Theoretical methods.

Our main objective is to get a description of the fragmentation processes of doubly charged molecule. In collision with swift ions the ionization process is much faster than the fragmentation. Auger decay after photoemission of a core electron also occur in a time scale shorter than fragmentation. Therefore, electron removal from the neutral molecule is sudden compared to the fragmentation time and one can safely assume that the initial geometry of the doubly charged molecule and the velocity of its nuclei just after ionization are the same as for the neutral.

To account for non-adiabatic effects during the fragmentation arising we have used Molecular Dynamic (MD) simulations within the Ehrenfest formalism, where the mean field potential energy

surface driving the nuclear dynamics is computed at the Time Dependent Density Functional Theory (TDDFT) level[17]. Initial electronic excited states are generated by removing two electrons from inner-shells orbitals[18] that are obtained using a DFT formalism, i,e, Kohn-Sham orbitals. In order to sample different initial ionization conditions two electrons are extracted form different kind of orbitals, for instances, centred in different regions of the molecule or with a different character (i.e. σ or π, localized or delocalized) allowing us to explore how the bond chemical environment affects the fragmentation process. TDDFT MD simulations are extremely expensive from the computational point of view, since the propagation of the equations for electrons requires the use of very short time steps (about 0.24 attoseconds) to ensure the conservation of the energy during the trajectory. After certain time it becomes in general possible to switch to ground-state Born-Oppenheimer (BO) MD to follow the evolution of the system that has become essentially adiabatic. To choose the correct time at which TDDFT MD can be switched to BOMD it is important to verify that both treatments produce the same trajectory in a given time interval. BOMD uses much longer time steps, of the order of 24 as, allowing to explore the evolution of the system up to hundreds of fs, where all the final fragments are produced. In the case that the two electrons are extracted from the HOMO, fragmentation can be studied using standard Car-Parrinello MD (CPMD)[19] from the very first instant after ionization. Due to the very high computational times involved in TDDFT MD it is unaffordable to consider extraction of two electrons form all the possible orbitals and only few cases can be selected. Moreover it is also difficult to consider double ionization from different orbitals, as the use of Local Spin Density approximation would further increase the computational times. TDDFT MD has been successfully applied to the study of the radiolysis of bulk water[18].

The implementation of the above mentioned methods used in our study are the ones corresponding to the CPMD code[20], which makes use of plane waves and periodic boundary conditions. In the case of uracil we have considered both the molecule isolated and in water solution, A cubic box of L= 19 Å was used for the isolated molecule and in the case of the simulation of aqueous solution each box (L=11.5 Å) contains one uracil molecule surrounded by 49 water molecules to reproduce the density of the liquid water [21]. In the plane wave basis a kinetic energy cutoff of 70 Ry was chosen. Core electrons are replaced by standard pseudopotentials of the Troullier-Martins form[22] and the exchange correlation energy is calculated using the generalized gradient approximation BLYP functional[23,24]. The initial atomic configuration is taken from an equilibrated trajectory of the neutral systems at 350 K using CPMD simulations. Atomic charges are evaluated using Bader analysis[25].

An alternative to CPMD to perform Ab inito MD has been proposed by Schlegel et al. in the Atom-Center Density Matrix Propagator (ADMP) method[26-28]. This formalism uses gaussian basis functions instead of plane waves and one-particle density matrix within the extended Lagrangian instead of the Kohn-Sham molecular orbitals.

As we will show CPMD and ADMP calculations are useful tools to explore possible evolution of the system when the internal energy increases. By increasing the temperature in the simulations it is possible to identify which are the most labile bonds and to have an estimation of the energy necessary to break them. Also these methods allow to identify potential reaction products that are difficult to foresee using chemical intuition, as doubly charged species often isomerise very rapidly leading to unexpected fragments.

3. Fragmentation of doubly charged Uracil in gas-phase and aqueous solution

In two recent studies[16,29] we have simulated the non-adiabatic fragmentation of uracil^{+2} in gas[16] and aqueous phases[29]. In the case of gas-phase calculations doubly ionization from five different initial orbitals were considered. The TDDFT MD calculation predicted dissociations that were

compatible with experiments in which uracil^{+2} was produced in collisions with 100 keV protons and fragments detected in multicoincidence[16]. Calculation not only reproduced the most intense signals in the spectrum, but also their shape by using the calculated velocities of each fragment. One important aspect of this study was to show how the chemical environment strongly affects the obtained fragmentation patterns. Figure 1 represents the fragmentation paths followed when two electrons are extracted from the two most inner orbitals (named as KS1 and KS2). Both orbitals have similar character (σ orbitals localized in C-O bonds) but differ in their chemical environments (between two N in the case of KS1 and between N and C in the case of KS2). As shown in Figure 1, not only the final fragments obtained are quite different, but also the systems evolve in a very different way. In the case of KS1 the first bonds that break are the C-N ones at different times (14 and 32 fs) and soon after (34 fs) the C_1-C_4 bond also breaks leading to three final fragments. One of them is the original CO bond from which electrons were extracted that is not broken. BOMD simulation started at 70 fs shows an isomerization of the NHCHCH fragment to produce a most stable CH_2CNH isomer. In the case of KS2 dynamics, on the contrary, the ionized C=O bond breaks in the early stages of the dynamics (4 fs) ejecting a neutral O. At longer times the remaining cycle breaks in three fragments, BOMD shows that the oxygen original attached to C_2 finally bonds to C_4. An important feature of both dynamics is that fragmentation start at very short times and that charge reorganization and localization at different atomic centres is nearly completed at 20 fs.

The two dynamics, starting from two-electron holes in exactly the same orbitals, have been also studied in the aqueous solution[29] and show completely different mechanisms (see Figure 2). Now in the case of ionization from KS1 the release of O (5.5 fs) is first observed, followed by H1 (85 fs) and H3 (116 fs) ejections. Within 100 fs, a neutral six membered ring is created, solvated, and stabilized in liquid water. At longer times, this cycle opens giving the final product shown in Figure 2. The ejected O reacts with surrounding water molecules to give H_2O_2. In the case of ionization from KS2 a simultaneous departure of the O4 and H3 atoms is first observed, followed by the departure of the H1 proton (37 fs), leaving a neutral ring. This structure is not stable and opens up leading to the molecule shown in Figure 2, which is stable in water solution and is maintained until the end of the simulation.

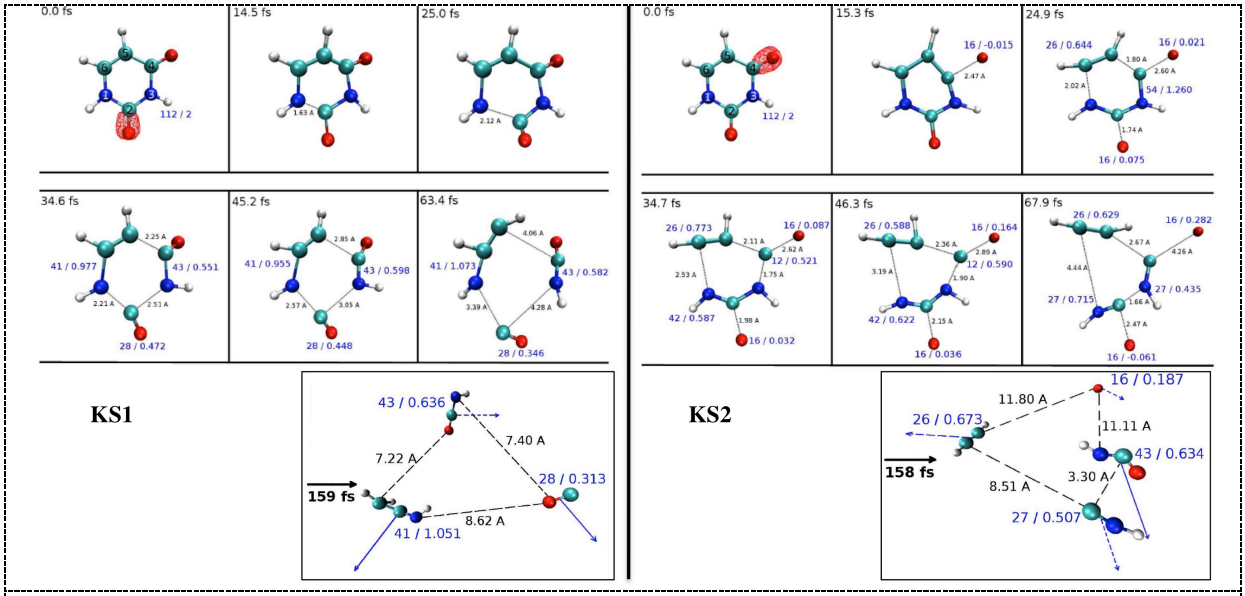

Figure 1. Snapshots for the TD-DFT MD trajectory after the ionization of uracil^{+2} in the gas phase from the two most inner orbitals Kohn-Sham orbitals (KS1 and KS2). At 70 fs calculations are switched to BOMD. Lower frames correspond to the end of the BOMD, Numbers indicate the mass/charge of each fragment and the internuclear distances (in Å)

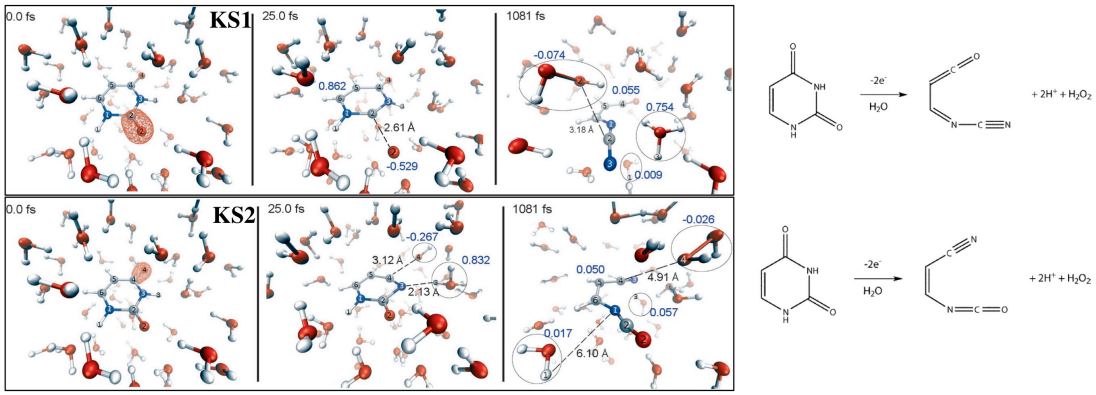

Figure 2. Snapshots for the TD-DFT MD + BOMD trajectories after the ionization of uracil^{+2} in the aqueous solution from the two most inner orbitals Kohn-Sham orbitals (KS1 and KS2). The switch from TDDFT MD to BOMD is done at 25 fs. Right hand side of the figure shows the final products.

The ejected O atom leads to the production of two OH radicals, which subsequently react and form H_2O_2 within 100 fs of dynamics, while the leaving protons lead to two solvated H_3O^+ ions.

A deeper insight of the role played by the solvent can be obtained by looking at the evolution of the electronic density given in ref. [29]. In gas phase 5 fs after ionization the entire ring appears to be affected by the ionization process and the charges are largely delocalized. In contrast, in liquid water at 5 fs the electronic hole is still located in the vicinity of the CO fragment. As a consequence, the dissociation is much faster and more located in the CO bond for liquid phase, while in the gas phase the whole ring breaks into three different fragments. Furthermore, the water molecules directly hydrogen bonded to uracil appear to be involved in the early charge reorganization, suggesting that hydrogen bonding plays a crucial role in localizing the charge.

Another important outcome of the liquid phase simulations is that they allow considering initial ionization from water molecules, either doubly ionization of a single water molecule or singly ionizing two water molecules. In this way it is possible to evaluate indirect radiation damage effects, for instance these simulations reveal the hydroxyl-mediated oxidation of uracil[29].

4. Fragmentation of doubly charged amino acids in gas-phase

The second type of biomolecules we have investigated using these techniques are amino acids. As building blocks of proteins they can be involved in radiation damage processes, but also the stability of doubly charged amino acids is a topic of interest in astrochemistry, since the enhanced reactivity shown by dications has been proposed as a mechanism to explain the formation of relevant new species in the interstellar media[30]. In recent years we have explored the fragmentation of α-amino acids (the natural occurring ones) but also β- and γ- amino acids where the amino (-NH_2) and carboxylic (-COOH) are separated by 2 or 3 carbon atoms respectively. Multicoincident measurements in both ion collision experiments, for instances Glycine with Xe^{25+} at 387 keV[31], γ-aminobutyric acid (GABA) in collision with 48 keV O^{6+} [15] or synchrotron radiation induced fragmentation in methionine[31] show rich spectra with a large variety of fragments produced.

To have an initial overview of the different accessible products it is necessary to performed extensive explorations of the Potential Energy Surface (PES). As an example, for the GABA molecule the PES corresponding to the dication is given in Figure 3. In this case one would expect that coulomb

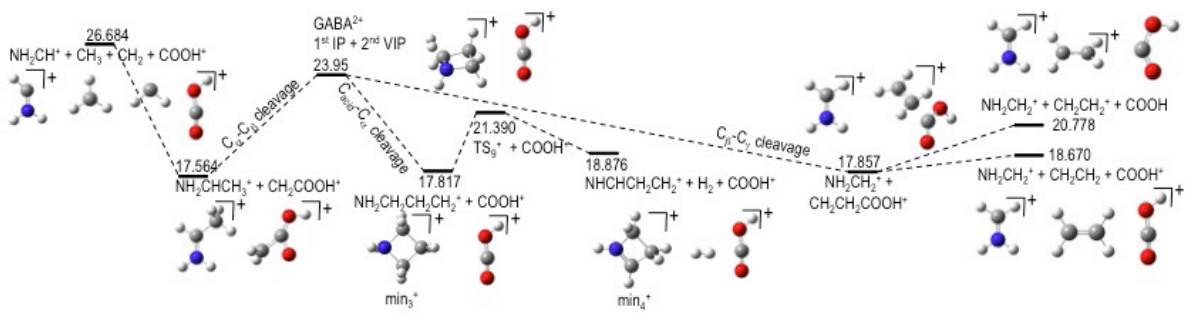

Figure 3. Fragmentation pathways for doubly positively charged GABA molecule. Numbers indicate relative energies (in eV) with respect the most stable isomer of the neutral molecule. Energies are obtained at the B3LYP/6-311++G(3df,2p) level using B3LYP/6-31++G(d,p) geometries and zero point energies corrections.

explosion would produce the breaking of one of the C-C bonds, leading to the possible charged fragments depicted in the figure. Isomerisation processes can also compete with direct bond breaking, for instance in Figure 3 we identify a four member ring dication to be a very stable specie that can play an important role and be one of the products observed in the experiments. It has to be emphasized that the number of possibilities that arise when considering isomerisation are very high, even for small amino acids, and are difficult to evaluate in a systematic way. One alternative to a complete exploration of the PES is to perform CPMD or ADMP calculations at different initial conditions. Figure 4, shows a summary of the results obtained after running of the order of one hundred ADMP dynamics with different initial internal energies for the GABA molecule. Breaking of the C-C bonds are the most frequent channels, but some channels involve isomerisation processes in some cases difficult to predict as, for instance, the formation of a 5-member ring shown in Figure 4.

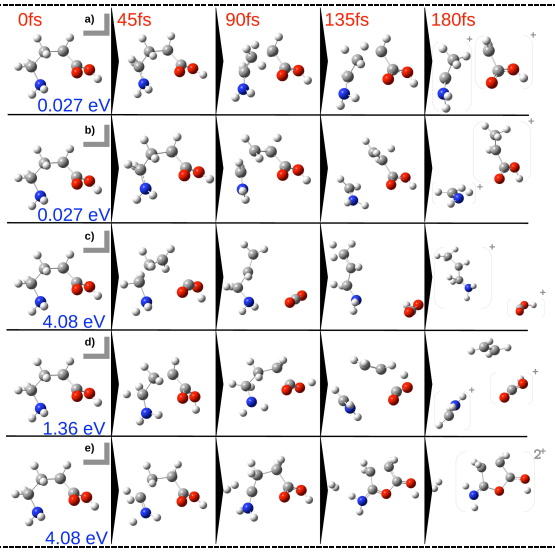

Figure 4. Snapshots of different reaction channels obtained at the ADMP level using different initial conditions. Only some of the fragmentations channels obtained are depicted. Coulomb explosions (channels a-d) are the most probable ones, but isomerisation and formation of stable doubly charged species (as channel e) are also obtained.

Although these calculations are useful to have an estimation of the possible fragments, the study of nonadiabatic fragmentations when electrons are extracted from inner shells requires the use of TDDFT MD. As a final example we present in figure 5 the reaction path obtained when two electrons are extracted from the Kohn-Sham orbital 5 of α-alanine (corresponding to a 2s orbital localized in the N). The complex dynamic observed (ejection of the CH_3 group followed by emission of a H^+ that recombines giving CH_4^+) is not obtained in any of the CPMD simulations performed at different temperatures. CPMD calculations predicts just the direct breaking of the molecules in $COOH^+$ + $CH_3CH(NH_2)^+$ and only at very high initial temperatures the formation of other fragments as $H_2CCH_2^+$. An interesting feature observed in α-alanine, and also in other amino acids, is that the times needed to break the bonds in TDDFT MD simulations are of the order of 50 fs, much longer that the times obtained in uracil. The greater flexibility of the bonds in amino acids that better allows a redistribution of the energy in vibrational modes seems to be at the origin of this finding.

5. Conclusions

Simulations of fragmentation dynamics of doubly charged biomolecules using Ehrenfest TDDFT and BOMD give results that are in good agreement with recent experiments in which the biomolecule is ionized in collisions with ions or by using synchrotron radiation. These simulations also allow to have a deeper insight in the dynamics of fragmentation of biomolecules at very short times, where electronic charge redistribution are taking place. In the case of uracil the environment strongly affects the response to ionization, explaining why fragments obtained in the gas-phase are very different from those obtained in aqueous solution. Simulations using less expensive MD techniques as CPMD or ADMP at different initial internal energies are useful tools to explore potential fragmentation channels, but TDDFT calculations are unavoidable to have a complete description of the fragmentation dynamics and to reproduce some of the channels observed experimentally.

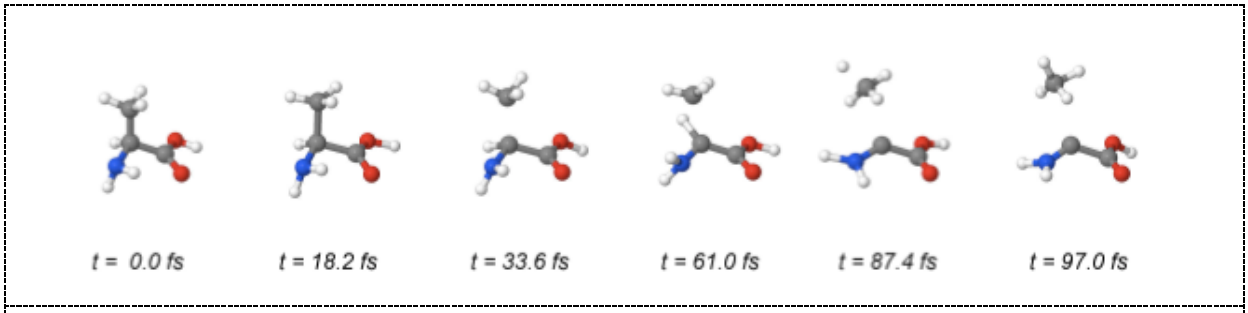

Figure 5. TDDFT MD trajectory for alanine when two electrons are initially extracted from the 2s orbital of N

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