Oxidative DNA Damage

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Ultrafast Damage Following Radiation-Induced Oxidation of Uracil in Aqueous Solution**

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Various types of ionizing radiation are commonly used in cancer therapy to irradiate tumors. In particular, hadron therapies have emerged as promising methods owing to their ability to deposit most of their energy in the Bragg Peak, at a well-determined depth inside the tumor (a few centimeters from the entry point, i.e. the skin).

There is strong evidence that severe biological effects may result from a class of complex DNA lesions induced by significant energy deposits in or near DNA,[1–3] producing either ionization of DNA components (direct effect) or chemical attack of DNA by radicals formed in the surrounding medium (indirect effect or quasi-direct effect when oxidation takes place in the hydration layer[4]). Major efforts have been dedicated during the past decades to developing experimental techniques able to determine the primary molecular fragments that result from oxidative damage to cellular DNA.[5]

The products formed and the influence of the sugar-backbone following single ionization of nucleic acids by UV (photon) irradiation in liquid water have been investigated.[6,7] The advent of femtosecond time-resolved laser spectroscopy has further enabled the investigation of early charge-transfer processes and elementary radical reactions in molecular liquids and solutions.[8] These femtolysis experiments have demonstrated the inadequacy of classical analysis for a proper representation of short-range coupling between non-equilibrium electrons and nascent water-decomposition products.[9,10]

In contrast, swift ions induce multiple ionizations along their tracks (about 10% of primary ionization events before reaching the Bragg peak). These multiple ionization events have been shown to induce irreparable DNA damage when produced in conjunction with low energy electrons.[11] The specific consequences of multiple ionization events have nevertheless received less attention and can hardly be discriminated experimentally in the condensed phase.[12]

From a theoretical point of view, molecular dynamics (MD) simulations are proper methods to get a detailed understanding of time-dependent chemical reactivity occurring in complex molecular systems. Among them, first-principle MD methods (classical nuclei quantum electrons) provide the most accurate description. While first-principle MD methods have been used to investigate reactions of radiolytic products with nucleotides,[13–15] they have rarely been applied to the chemical reactivity of ionized nucleobases in a water environment. Herein, we show a microscopic model of the early stages (femtosecond up to picosecond) following radiation-induced ionization of nucleic acid building blocks in aqueous solution.[16] We will focus herein on the oxidation of uracil (an RNA base, but also a good model for pyrimidine bases) embedded in liquid water.

We have developed and applied a first-principle MD method to generate and control ionization events and follow the early stages of the Coulomb explosion, subsequent to the double ionization of a molecule immersed in liquid water. This method is based on time-dependent density functional theory (TDDFT) MD in which effective molecular orbitals (MOs) are propagated in time.[17] The ionization process is obtained by removing electrons from selected MOs (see [18–20] and the Supporting Information for a detailed description of this method). Herein, we consider doubly ionized uracil produced in aqueous solution and show, by comparison with the gas phase results,[18] that the local liquid environment plays a crucial role in the observed dissociation. All nuclei are treated classically. This is expected to be a good approximation because the observed proton transfers are very fast, so that quantum effects such as tunneling are negligible.[21]

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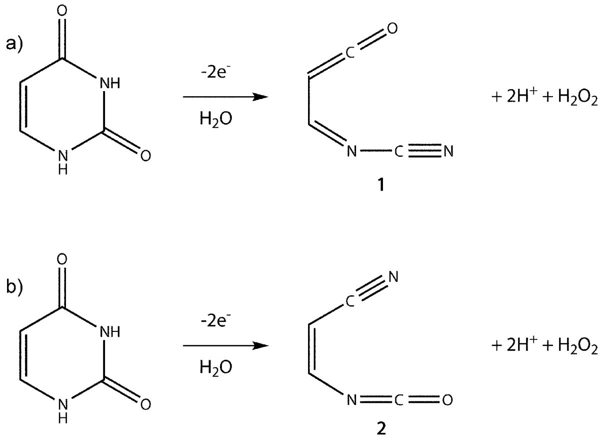
We have generated five TDDFT-MD initial ionization conditions. The first two were chosen to explore the most energetic effects of direct double ionization of uracil. They correspond to the removal of two electrons from the deeper MOs (9a′ and 10a′) which are the σ bonding orbitals of the C2=O2 and C4=O4 groups of uracil.

To investigate possible indirect oxidation pathways, the three other initial conditions correspond to the ionization of surrounding water molecules: 1) two of these conditions were generated by double ionization of a single water molecule, removing two electrons from the 2a1 and 1b2 MOs; 2) the other condition was initiated from two singly oxidized water molecules. A local spin-density representation was used in this case. Oxidation of two water molecules is the most probable event, since single ionization produced by multiple collisions in swift ion tracks (before the Bragg peak) occurs for molecules that are separated by 0.5 nm on average (Supporting Information, Table 1S).[19]

All these reactions occur far from equilibrium, involving highly excited electronic states and kinetic energies of atoms that can reach a few eV, within the scope of the TDDFT-MD simulation. In the Supporting Information, we report the reference Gibbs free energies as a guide to understand the stability of the products formed in the later stage of the dynamics.

When ionization proceeds from the 9a′ uracil MO, the release of O2 (5.5 fs) is first observed, followed by H1 (85 fs) and H3 (116 fs; Figure 1). Within 100 fs, a neutral six-membered ring is created, solvated, and stabilized in liquid water. Such a ring was found to be energetically stable in solution (Supporting Information, Table 2S and Figure 1S). Nevertheless, at longer times, this cycle opens along the N3–C4 bond leading to product **1** (Scheme 1 a) and remains open until the end of the simulation, which lasts about one picosecond. This dissociation of the molecule embedded in water differs from that observed in the gas phase, where doubly ionized uracil (Ura^{2+}) fragments into three pieces (CO, HNCO^{+}, HNCHCH^{+}) when ionized from the 9a′ MO.[18]

To further clarify the role played by the solvent on the subsequent dynamics of Ura^{2+}, we have analyzed the time evolution of the electronic density difference with respect to the structure immediately after ionization during the first 5 fs of dynamics (Figure 2). In the gas phase, the entire ring appears to be affected by the ionization process and charges are largely delocalized. In contrast, in the condensed phase the electronic rearrangement is mainly confined to the nearest neighbors of the C2–O2 pair. The better ensuing localization of the hole on C2–O2 in liquid water is probably at the origin of the faster dissociation; in the liquid phase, the O2 atom dissociates in 5 fs, while in the gas phase, dissociation takes 30 fs. While the dissociation in liquid involves only the atoms of the CO fragment, the whole cycle is involved in the gas phase dynamics, with the cycle fragmenting into three



Scheme 1. Oxidation reactions of uracil immersed in liquid water after double oxidation from a) 9a′ and b) 10a′ MOs.

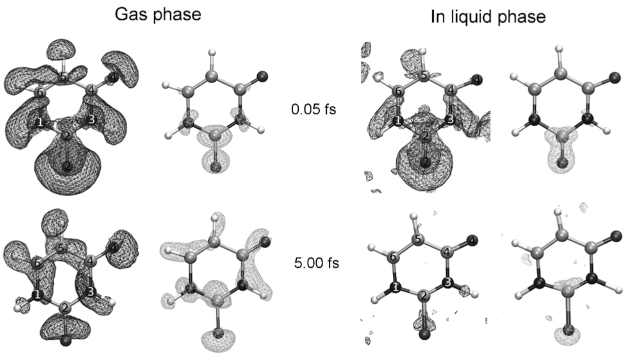


Figure 2. Difference between the electronic density at $t=0$ fs and at t. Isovalues correspond to one electron (light gray contour) and one hole (dark gray contour) at $t=0.05$ fs and two electrons (light gray contour) and two holes (dark gray contour) at $t=5$ fs.

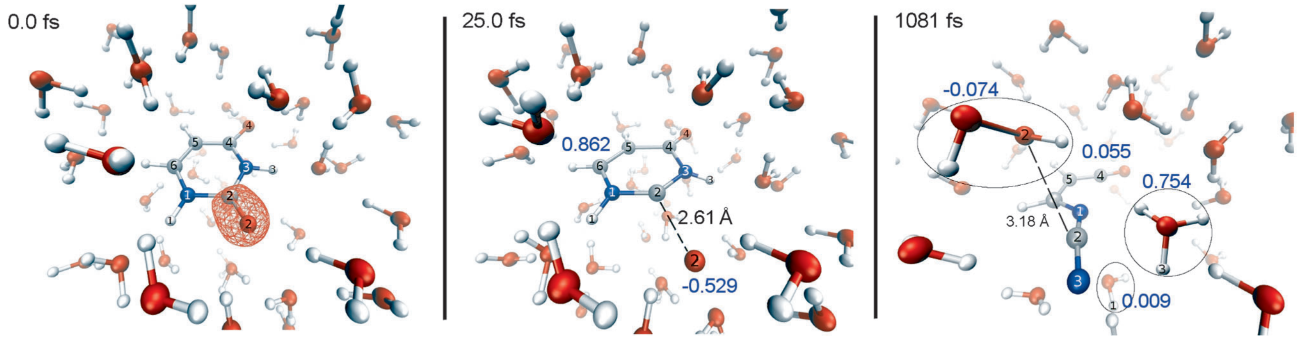


Figure 1. TDDFT-MD trajectory initiated from an electronic configuration where two electrons were removed from the 9a′ MO of uracil (localized on C2=O2, red contour; left, $t=0$). C gray, N blue, O red, H white.

pieces. Furthermore, the molecules directly hydrogen bonded to uracil appear to be involved in the early charge reorganization. This suggests that hydrogen bonding plays a crucial role in localizing the charge around C2—O2. Building of a hole away from the region of the 9a′ MO, leads to an electronic repopulation of C2 and O2 atoms. This excess charge is not localized in between these two atoms but in the region corresponding to an antibonding orbital, particularly at 5 fs.

When ionization is performed from the 10a′ MO (localized on C4=O4, Figure 3), a simultaneous departure of the O4 and H3 atoms (5.2 fs) from Ura^{2+} 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 along the C2—N3 bond (after approximately 41 fs) leading to molecule **2** (Scheme 1 b), which is stable in water (Supporting Information, Table 2S and Figure 2S) and is maintained until the end of the simulation. Molecule **2** is very similar to **1**, where the terminal O and N atoms are interchanged. Their Lewis structures (Supporting Information, Scheme 1S) show that they differ in the alternation of double and single bonds in the conjugated systems, making **2** more stable than **1** (Supporting Information, Table 2S). In the gas phase, the same initial Ura^{2+} state (10a′ MO) leads to completely different dynamics with the formation of four fragments.[18] Here, the ejected O atom leads to the production of two $^{\bullet}$OH, which subsequently react and form H_2O_2 within 100 fs of dynamics, while the leaving protons lead to two solvated H_3O^+ ions.

The two dynamics assessing the indirect effect of oxidation by double ionization of one single water molecule (2a1 and 1b2 MO) result in the Coulomb explosion of H_2O^{2+} (3 fs), as in the pure liquid phase.[20] The second indirect ionization scenario, where two different water molecules are singly ionized from the 2a1 MO, leads to a hydroxyl-radical-mediated oxidation of uracil at the C5 atom (Figure 4 and Scheme 2), a well-known radiation-induced reaction in aqueous thymine.[5,22] The reaction scheme is complex: the uracil molecule in the vicinity of H_2O^+ first gets oxidized by charge transfer, and subsequently, in about 30 fs, the same water molecule binds at C5 of uracil, expelling one hydrogen atom from the water molecule (Supporting Information, Figure 3S); this is consistent with uracil having a lower ionization potential than water. The resulting OH attachment leads to the formation of a 5-hydroxy-5-hydrouracil radical (5-OH-5-H-Ura). Such Ura$^+$ reaction with water was described in [7]. In the next 50 fs, the 5-OH-5-H-Ura radical transfers one electron to the other initial H_2O^+.

The endpoint of this trajectory is thus the formation of a cation **3** (Scheme 2), which is a protonated 5-hydroxyuracil. From **3**, two further reactions may occur, leading to 5-hydroxyuracil (**4**, Scheme 2) through deprotonation, or to a glycol (**5**, Scheme 2). These reactions were not observed in our sub-picosecond trajectories but have been identified in radiolysis experiments of solvated uracil.[22] Oxidized nucleobases represent one of the main classes of damage induced in DNA by ionizing radiation. The main reaction of $^{\bullet}$OH with the nucleobase is well known to consist in the addition of a hydroxyl group to an unsaturated bond (C5—C6 for uracil and thymine),[5] similar to the reaction observed herein after oxidation. Note that a similar hydroxylation reaction has been measured after single ionization of a base in the presence of the sugar-phosphate moiety.[6]

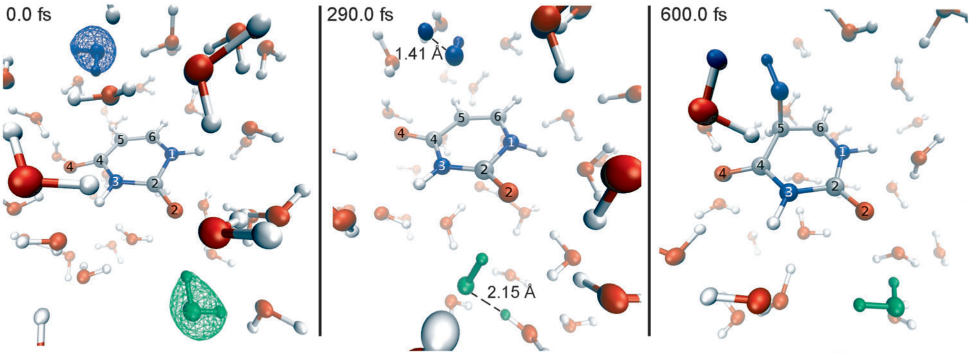


Figure 4. Uracil hydroxylation. Singly oxidized water molecules from the 2a1 MO (blue and green contour) leading to attachment of one OH on the C5 atom of uracil. C gray, N blue, O red, H white.

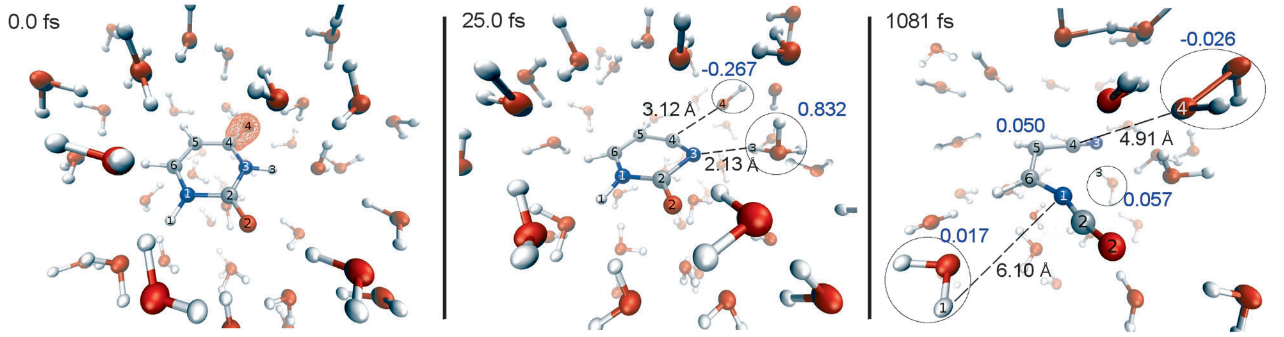
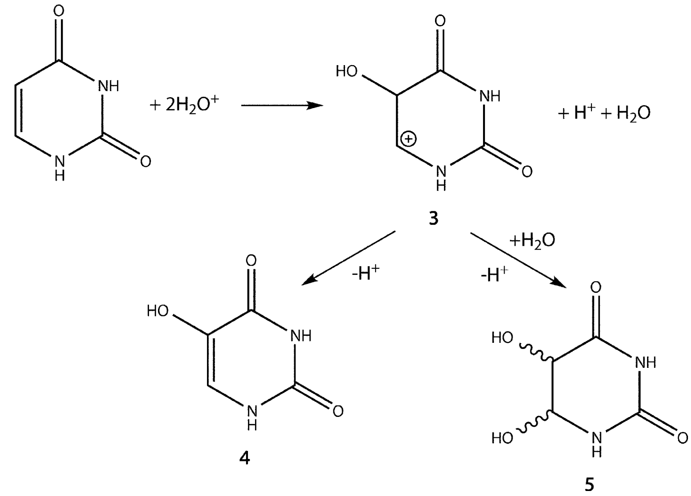


Figure 3. TDDFT-MD trajectory initiated from an electronic configuration where two electrons were removed from the 10a′ MO of uracil (localized on C4=O4, red contour; left, $t = 0$). C gray, N blue, O red, H white.



Scheme 2. Oxidation reaction of solvated uracil when two surrounding water molecules are singly oxidized.[5,22]

The products of direct oxidation of uracil (**1**–**2**) are thus very different from the products observed when water is oxidized first (**4**–**5**). While **4**–**5** are the same products as those observed in the chemical oxidation of uracil (or thymine), molecules **1**–**2**, on the contrary, are the result of ring opening without oxidation of any atoms of the ring (the ejected oxygen is the oxidized species). Molecules **4**–**5** are typical of oxidative stress and lead to well-identified aberrations, in particular the AT-CG mutation.[23] While we may speculate that there exists biological mechanisms protecting DNA from these oxidized water derivatives in solution, the situation is very different for molecules **1**–**2**, which appear characteristic of the direct double ionization of uracil. Experimentally, it has been shown that local DNA double oxidation (a carbon K-shell ionization plus Auger process) implies more complex damage than other radiolysis processes.[11] The different reaction schemes identified herein, suggest that these damages may be very different chemically, with more exotic structures involved than those obtained from oxidation of DNA under milder conditions.

To summarize, direct and indirect ionization effects have been explored, using TDDFT-MD simulations, where the base and the solvent are explicitly treated at the same level of theory (DFT/BLYP). We have shown that the response of the uracil molecule to ionization is strongly affected by its environment and cannot be simply inferred from the gas-phase patterns. In addition to the specific damages induced to uracil, charge transfers from uracil to water lead to the formation of radicals (OH, O, and H_2O_2) within a few hundred femtoseconds, in contrast with the general assumption that these radicals can only be generated through a much slower (indirect) effect. Interestingly, we have also found "fast" indirect processes, such as the hydroxylation of uracil in the C5 position, resulting from the reaction with a singly ionized water molecule without the formation of OH radicals. As a last note, water is often presented as protecting DNA,[24] thus preventing or minimizing DNA fragmentation from direct ionization events. Our simulations suggest that this argument should be revisited.

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