Femtoradical events in aqueous molecular environments: the tenuous borderline between direct and indirect radiation damages

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Abstract. The complex links existing between radiation physics and radiobiology concern the complete understanding of spatio-temporal events triggered by an initial energy deposition in confined spaces called spurs. Microscopic radiation effects (photons or relativistic particles) on integrated biological targets such as water “the solvent of life” and biomolecular architectures (DNA, histones, enzymes) cannot be satisfactorily described from an absorbed dose delivery profile or a linear energy transfer (LET) approach. Primary radiation damages on biological targets being dependent on the survival probability of secondary electrons and short-lived radicals inside nascent nanometric clusters of ionisation, a thorough knowledge of these processes require the real-time probing of early events on sub-micrometric scale, in the temporal range $10^{-15} - 10^{-10}$ s. Major strides concern early water damages: primary water cation formation ($H_2O^+$ or positive hole), concerted electron-proton couplings, attachment dynamics of $p$-like excited prehydrated electron on biomolecule, short-lived radical pairs involving water-bridged radical $OH^-$ and hydronium ion $H_3O^+$. The deactivation frequency of electron-radical pairs is comparable to an $H-OH$ deactivation of excited water molecules ($\nu_{H_2O^*} \sim 0.33 \times 10^{13} \text{ s}^{-1}$). These short-lived events take place in the prethermal regime of delocalized secondary electrons and represent a tenuous borderline between direct and indirect molecular damages.

1. Introduction
It is commonly admitted that the initial spatial distribution of energy deposition following the interaction of ionizing radiations with sub-cellular and biomolecular targets is decisive for the prediction of long time radiation damages. The fundamental importance of understanding primary ionising radiation effects on water, “the lubricant of life”, is emphasized in fields such as radical chemistry of proteins, DNA single and double strand breaks, molecular repair, radiotoxic lesions leading to apoptosis, radiation biology and finally radiotherapy [1-5]. The deleterious consequences of ionizing radiations (photons or particles) on biomolecular targets can dependent on the nature of radio-induced defects triggered within solvation shells containing free or interfacial water (figure 1). The distinction between direct and indirect ionizing radiation effects becomes tenuous when inhomogeneous spatio-temporal events are considered at the local order.

Following an energy deposition, a thorough knowledge of elementary phenomena involved in early water and biomolecule radiation damages require the real-time observation of transient events. Time dependent molecular excitation, ionization and generation of very reactive radicals are attracting
growing experimental and theoretical interests for oxidoreduction reactions relevant to ultrafast radiation damages in biomolecular environments [6-8].

**Figure 1.** Synthetic representation of multiple electron trajectories taking place in the vicinity of a biomolecular target, following a water defect triggered by ionizing radiations.

2. Primary radiation damages of water molecules: a spatio-temporal investigation

The wide impact of ionizing radiations with water molecules concerns multiphotonic energy deposition, inelastic interactions of high-energy radiations (relativistic particles, X or γ rays), electronic or vibrational excitations and ionizations processes. Primary physicochemical steps take place in the prethermal regime, i.e. in less than $1 \times 10^{-12}$ s and involve multiple radical events in confined spaces. With the intensive development of ultrafast laser techniques and high time-resolved spectroscopy, major strides have been performed on the investigation of primary water defects propagation, ultra-fast electronic dynamics and early recombination processes on the time scale of molecular motions [9-11]. An energy deposition via a two-photon excitation with femtosecond UV pulses ($2 \times 4$ eV for instance) induces early water defects (formation and migration of a positive hole $H_2O^+$) and multiples non-equilibrium configurations of trapped electron (quasi-free delocalised electron $\{e_{-qf}\}$, p-like excited prehydrated electron $\{e_{p}\}$, electron-radical pairs and hydrated electron ground state $\{e_{s}\}$). These states are populated in less than $5 \times 10^{-13}$ s and are separated by different energy gaps in the range 1 - 1.7 eV (figure 2). The primary water molecular cation $H_2O^+$ reacts with surrounding water molecules via an ultrafast ion-molecule reaction, equation (1). This early event occurs in less than $10^{-13}$ s, yielding a strongly oxidizing OH radical and hydronium ion $H_3O^+$ (hydrated proton). It is likely one of the fastest that occur in polar molecular solvents and represents an ideal case to learn more about ultrafast defect migration and proton transfer in water environment [9].

$$\begin{align*}
\text{H}_2\text{O} \ldots \text{H}_2\text{O} \quad &\xrightarrow{\text{Ionisation} < < 10^{-13} \text{s}} \quad \text{H}_2\text{O}^+ \ldots \text{H}_2\text{O} \\
\text{nH}_2\text{O} &\xrightarrow{\text{v_0}: 10^{13} \text{s}^{-1}} \text{OH}^+\text{H}_3\text{O}^+ \\
\text{nH}_2\text{O} &\xrightarrow{\text{H}_2\text{O}} \text{H}_2\text{O} 
\end{align*} \quad (1)
$$

A structured environment induced by hydronium ion ($H_3O^+_\text{aq}$) and hydroxyl radical ($OH^+_\text{aq}$) can be created before that an excess electron gets its equilibrium state. From a first excitonic state ($\sim 6.5$ eV), low dissociation channel initiates the formation of solvent bridged three-bodies complex [$OH^+\ldots e^- \ldots H_2O^+$]. This channel would compete with a high energy pathway ($\sim 8$ eV) involving an $A^-$ state (1b1 $\rightarrow$ 3a1 for instance). At the same energy level that an excited p-state electron a second excitonic state would contribute to a direct electron delocalisation in the vicinity of the water conduction band [12].
Considering the equation (2) for which $\eta_t$ represents the trap density ($\sim 4.4 \text{ m}^{-1}$ [13], $\tau_t$ the electron trapping time ($1.1 \times 10^{-13} \text{s}$ in liquid water [14]), $v_{th}$ the electron velocity ($\sim 1.05 \times 10^7 \text{ m} \text{s}^{-1}$), an estimated electron trapping cross section of $40 \text{ Å}^2$ would correspond to a spatially extended electron-trap radius of $4 \text{ Å}$. This value agrees with the radius of “p” like state excited electron and is larger than the estimated radius of “s” like ground state hydrated electron, $r \sim 2.3 - 2.8 \text{ Å}$ [15].

$$\sigma_t = \frac{1}{\eta_t \left( \tau_t \cdot v_{th} \right)}$$  \hspace{1cm} (2)
(reduction potential $\sim +2.35$ V). The early spatial distribution of water products fluctuates at the local order i.e. few molecular radius. Their interaction dynamics depend on the microscopic properties of water molecules, lifetime of prototropic entities and initial spatial distribution functions (figure 3).

Two well-defined electron recombination pathways are illustrated in figure 4. When an excess electron is directly trapped in the structured hydration shell of hydronium ion ($\text{H}_3\text{O}^+$) or hydroxyl radical (OH$^*$), the initial separation distances are shorter than the Onsager radius ($R_c \sim 7$ Å in water). These transition states correspond to water bridged radical pairs whose a monoexponential near-infrared relaxation process ($\tau = 340$ fs) leads to the hydrated hydrogen atom $\text{H}_3\text{O}^+$ and hydroxyl ion $\text{OH}^-$, equation (3). The high deactivation frequency of electron-radical pairs ($\nu_2$) is comparable to the estimate of excited water molecules $\text{H}_2\text{O}^*$ deactivation $\nu_{\text{H}_2\text{O}}^* \sim 0.33 \times 10^{13}$ s$^{-1}$.

For initial separation distances $R_i$ longer than the Onsager radius (figure 3), independent pairs $e^-..\text{OH}^*$, $e^-..\text{H}_3\text{O}^+$ predominate and execute a 1D walk before undergoing an efficient recombination, equation (4). The time dependence of $e^-$, escaping geminate recombination is defined by the equation (5).

$$
\text{H}_2\text{O} \xrightarrow{\text{Ionising Radiation}} \left\{ \begin{array}{c} \text{e}^-, \ \text{OH}^*, \ \text{H}_3\text{O}^+ \\ \text{nH}_2\text{O} \end{array} \right\} \xrightarrow{\text{Ultra-fast recombinations}} \left\{ \begin{array}{c} \text{OH}^-, \ \text{H}_3\text{O}^+ \\ \text{nH}_2\text{O} \end{array} \right\}$$

$$
e^-_s(t) = \int_{-\infty}^{t} \text{dn}_e \left( t' \right) \left\{ 1 - \gamma + \gamma \text{erf} \left( \frac{T_J}{t-t'} \right)^{1/2} \right\} \text{dt'}$$

with $\text{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-u^2} \text{du}$. This analytical solution contains two adjustable parameters: a 1D geminate recombination time $T_J$ and a recombination probability $\gamma$. The least-squares fit of the signal dynamics probed at 1.72 eV 20 nm gives $T_J : 1.2 \pm 0.1$ ps and $\gamma : 0.55 \pm 0.05$. The random 1D walking
electrons explain by itself the absorption signal dynamics in the 0 - 150 ps range and greatly satisfies the time-dependence $1/(t)^{1/2}$ of a dispersive recombination process. The incomplete signal decay is assigned to long-lived 1s-state of hydrated electrons (ground state).

From isotopic substitution experiments, it is emphasised that energy vibrational modes of water molecules and nuclear motions of protonated hydrates influence electron-proton coupling. The complete understanding of this elementary process between two quantum entities needs to ascertain whether the dynamics of electron-proton couplings are dependent on the initial electron-hole pair distributions, the multiple configurations of hydrated proton and/or intracomplex structural changes within a hydrogen bond network. A limit case involves short-range electron-proton couplings for which $\text{H}_2\text{O}^+$ undergoes one jump (1D motion by a finite process). For a diffusion coefficient expressed as $D \sim \lambda d^2/6$ and an experimental jump frequency $\lambda = 1/T_j = 0.5 \times 10^{12} \text{ s}^{-1}$, the initial proton jump distance would be around 2.8 Å. After several 1D jumps, typically 2-5, electron-radical pairs move away and a dispersive recombination dynamics prevails. During a very time range (less than $5 \times 10^{-12}$ s), highly reactive water bridged three-body complexes $[\text{OH}^-\cdot\cdot\cdot\text{e}^-\cdot\cdot\cdot\text{H}_2\text{O}^+]$ can potentially participate to direct ultrafast biomolecular damages triggered by ionising radiations [16].

3. **Ultrafast low energy electron attachment on a biomolecule**

Radiation induced radical events with disulfide biomolecules represent an important domain of oxidation-reduction reactions [17-19]. An electron attachment leading to an anionic radical RS$^-$:SR$^+$ contributes to a bond breaking phenomena with the formation of thyl radical RS° and RS$^-$ ions. Because disulfide radical anions regulate the functional properties of enzymatic systems, proteins or macromolecular complexes, disulfide molecules can be used as sensors of early molecular damages triggered by a direct attachment of a low energy electron (figure 5).

![Figure 5. Ultrafast infrared dynamics of a 2p-state excited electron attachment on disulfide molecule (cystamine) embedded in aqueous biomimetic organized cationic assemblies CTAB/PTH (phenothiazine). The consequence of a femtosecond electron attachment on the 1s-like ground state level is probed at 1.72 eV.](image-url)
The reactive center of oxidized glutathione (cystamine) is investigated in organized biomimetic systems. A delocalised electron state (2p excited state) is prepared by femtosecond two photon excitation (2 x 4 eV) of phenothiazine molecule embedded in the non polar core of cationic CTAB micellar systems [6]. Its ultrafast attachment on a disulfide biomolecule, equation (6), is probed in real-time by femtosecond infrared spectroscopy, considering the bond-bound/bound-unbound transition of the 2p state in the bottom of the conduction band (figure 2).

\[ (\text{RSSR} + \text{e}^{-}_{2p\text{ state}})_{\text{H}_2\text{O}} \xrightarrow{\text{Ultra–fast electron attachment}} (\text{RS} : \text{SR})_{\text{H}_2\text{O}} \]  

(6)

The figure 5 reports ultrafast attachment dynamics of a 2p excited electron on disulfide biomolecule. Femtosecond infrared dynamics probed at 0.99 eV substantiates a direct p-like electron attachment on the disulfide bridge. With a characteristic time \( T_{\text{REAC}} = 160 \pm 20 \) fs, the radical formation \( \text{RS} : \text{SR} \) takes place before a complete water caging relaxation of excess electron. The direct consequence of this sub-picosecond radical process on the initial yield of fully hydrated electron (s like ground state) is clearly observed by visible spectroscopy at 1.72 eV. The reaction radius of cystamine \( r_{\text{eff}} \) for an ultrafast electron attachment has been estimated from the probability \( P_{\text{Reac}} \) that an early IR electron transfer occurs inside the spherical volume of aqueous cystamine molecule, equations 6, 7.

\[
P_{\text{Reac}} = 1 - P_{p\rightarrow s} = 1 - \exp \left( -\frac{4\pi r_{\text{eff}}^3}{30^3} \right)
\]

(7)

\[
r_{\text{eff}} = \left( \frac{3\ln \left(\frac{1}{P_{p\rightarrow s}}\right)3\times10^3}{4\pi [\text{RSSR}]^3} \right)^{\frac{1}{3}}
\]

(8)

Figure 6. Effect of a direct attachment of p-like excited electron with a biomolecule (cystamine). The anti-bonding electron decreases the energy level of a native two-center-three-electron bond (2\(\sigma/1\sigma^*\)).
Experimental parameters $\alpha_{i}^{\omega T}$ represent the spectral contribution of an electronic state «i» at 0.99eV. The subpicosecond competition occurring between an ultrafast electron attachment on cystamine and a non-adiabatic $p \rightarrow s$ relaxation is defined by the high branching ratio $P_{\text{Reac}}/ P_{p \rightarrow s} \sim 0.38$. In these conditions, the reaction radius of cystamine $r_{\text{eff}}$ for an ultrafast attachment of excited electron is 10 Å (figure 6).

An early molecular damage of cystamine triggered by a well defined electron quantum state would involve some distortions of p-like electron orbital in the vicinity of a sulfur-sulfur bond. The subpicosecond lifetime of IR ($e_{p}$) represents a temporal limit for that an early rearrangement of a S-S bond energy facilitates an electron tunnelling through a potential barrier separating the IR p-state electron of an unpaired electron within a native RS$^-$/SR$^-$ radical anion. The antibonding electron contributes to a weakening and stretching of a two-center-three electron bond ($2\sigma/1\sigma^*$ bond). The decrease of the transition between the uppermost occupied lone pair representing the $\sigma$ energy level disturbed by a nonbonding sulfur electron and a singly occupied sulfur-sulfur $\sigma^*$ orbital can lead to a delayed S$^-$/S bond breaking in the sub-nanosecond range [17,18,20,21,22]. This femtosecond investigation demonstrates that ultrafast low energy electron attachments trigger direct biomolecular damages in the prethermal regime, before that significant water caging effects take place.

4. Concluding remarks

4.1. Spatio-temporal radiation events in ionisation clusters

The implication of secondary electrons during biomolecular damages triggered by ionising radiations (ultrashort high laser power, relativistic particle or X-rays), are crucial but their real-time observation in ionisation clusters remains difficult. One challenge concerns the complete understanding of spatio-temporal events triggered by an initial energy deposition inside confined clusters of ionization and evolving over several orders of magnitude, typically from femtosecond and sub-micrometric scales. During the interactions of relativistic particles with biological environments, confined ionisation clusters formation (spur) needs an energy deposition of about 20 eV. Due to the incertitude principle, equation (9), the fastest ionising events would take place in less than $0.33 \times 10^{-16}$ s.

$$\Delta t.\Delta E = h = 6.6 \times 10^{-16} \text{ eV.s}$$  \hspace{1cm} (9)

An incertitude principle on the particle position must also be considered during a spur formation $\Delta x = h$ with $p$ the momentum of the particle and $\Delta p = \Delta E/u$ if $u$ represents the particle velocity ($\approx 3 \times 10^{10}$ cm s$^{-1}$). From these two incertitude equations, an expression of $\Delta x$ can be extracted:

$$\Delta x = h \cdot \frac{u}{\Delta E}$$  \hspace{1cm} (10)

Indeed, an energy loss of 20 eV by a relativistic particle occurs on $10^{6}$ cm ($\approx 100$ Å). As primary radiation damages are dependent on the survival probability of secondary electrons and early radicals produced from water molecules and biomolecular targets, a thorough knowledge of these processes inside nanometric clusters of ionization requires real-time probing of early events on sub-micrometric scale. In the temporal range $10^{-15} - 10^{-10}$ s, this domain concerns high-energy radiation femtochemistry for which, as previously shown in sections 2 and 3, prethermal events can be probed in real-time [11].

4.2. Secondary electrons in native tracks and early biomolecular damages

Laser plasma accelerators providing shorter particle bunches [23] open exciting opportunities for the investigation of the course of ultrafast elementary ionising events. The qualities of short relativistic
particle bunches would foreshadow the development of spatio-temporal radiation chemistry and biology. The real-time investigation of relativistic particle interactions with biomolecular targets opens exciting opportunities for the sensitisation of confined environments (groove of DNA, protein pockets) to ionising radiation on the time scale of molecular motions, i.e. angstrom or sub-angstrom displacements. Recent femtolysis experiments (Femto-second radio-lysis) of aqueous targets performed with ultra short relativistic electron bunches produced by laser plasma accelerators (energy ~ 5-15 MeV) give new insights onto the early behaviour of secondary electrons in the prethermal regime of nascent ionisation clusters [11, 24]. These events concern the ejection, relaxation and hydration of excess electrons leading to the ubiquitous reducing radical: the fully relaxed hydrated electron. Its quantum character provides a unique probe to explore ultrafast couplings with the positive water hole H$_2$O$^+$ or water products. These pioneered femtolysis works emphasize that the early hydrated electron yield at t ~ 5 ps is higher than predicted by calculations using classical stochastic modelling of irradiated water molecules and underline the pre-eminence of specific quantum effects during ultra-fast secondary electron relaxation. In the MeV domain, the most promising developments would concern the real-time investigation of early radical events in the radial direction of relativistic particle beam [24,25].

In confined aqueous spurs, the ionisation density and early radical distribution represent major factors of biological radiation effectiveness. Regarding the prethermal regime, we address open questions on ultrafast radiation events that assist direct/indirect molecular damages of living matter (figure 7). The contribution of secondary electrons to direct water-mediated radical processes and bond breaking is particularly important for macromolecular systems such as DNA. Indeed, time-resolved laser studies devoted to ultrafast electron reactions with biomolecules whose disulfide biomolecule and derivates of deoxyuridines, potential radiosensitizing agents, confirm the crucial role of short-lived p-state hydrated electron in elementary univalent reductions [24, 26].

![Figure 7](image)

**Figure 7.** Open questions concerning the contribution of ultrafast DNA radiation damages following energy deposition by X rays or relativistic particles. The interplay between single or double strand breaks triggered by early oxidative OH$^-$ radical attacks and ultrafast low energy electron attachment remains to be clarified.

High energy femtoradical chemistry opens exciting opportunities for the sensitization of confined environments (protein pockets, groove of DNA) to ionizing radiation, for which target volumes of mass per area in size of about $1 \times 10^6$ g cm$^{-2}$ correspond to 100 Å at a density of 1.0 g cm$^{-3}$. This domain would foreshadow the development of new applications in radiobiology, particularly for the real-time nanodosimetry using well-defined quantum states of short-lived radicals.
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