O₂ and glutathione effects on water radiolysis: 
a simulation study

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Abstract. We present a MC simulation of water radiolysis when O₂ and glutathione are present in the solution. Our simulation is based on the continuum approximation proposed by Green and co-workers'. We investigate in particular the sensitivity of the yield of HO₂⁺/O₂⁻ to the concentration of O₂ and glutathione and to the LET of the ionising radiation. We demonstrate that the production of HO₂⁺/O₂⁻ is highly sensitive to these parameters and that the variation of their yield with the O₂ concentration shares remarkable similarities with the O₂ effect reported for biological cell damage efficiency.

1. Introduction
The effects of ionizing radiation in living cells depend on many parameters. Among other factors, oxygen pressure plays a significant role by enhancing cell sensitivity to low-LET radiation. In 1953 Gray et al [1] highlighted a correlation between the oxygen concentration and the radioresistance of hypoxic tumors to low-LET irradiation. An issue addressed by numerous studies [2-5] was then to define an “oxygen enhancement ratio” (OER) in order to qualify the cell radiosensitivity as a function of the supplied oxygen pressure (PO₂). The OER was defined as the ratio of the dose required in hypoxic conditions to the dose required at a normal pressure of oxygen, to produce the same biological effect. A compilation by Hall [5] of various experimental data pointed out an increase of the OER with oxygen pressure and a saturation at a pressure of about 30 mmHg. Experiments with neutrons [1] and alpha particles revealed a strong reduction of the oxygen effect with increasing the LET of the radiation. Decreasing the OER and increasing the RBE (Relative Biological Efficiency) were keys to the development of neutron therapy and, more recently, hadrontherapy [6-9]. It is also known that antioxidants could influence the oxygen effect. Indeed the oxygen effect was observed lower in glutathione-deficient cells [10-12]. Glutathione (GSH) is one of the most important antioxidant in the cell. Although the impact of antioxidant and oxidant in the oxygen effect is clear and has been known for a longtime, the mechanisms are still unclear.

The purpose of this work is to quantify by Monte Carlo simulations the influence of an oxygen concentration and an antioxidant concentration in the process of water radiolysis. While the production of hydroxyl radicals by water radiolysis is generally suspected to play a key role in the formation of damage in the important structures of the cells (DNA, membrane…), we will instead focus on the
production of HO$_2^*/O_2^-$/O$_2^-$ radicals. Indeed we shall see that the most important effect of the dissolved oxygen molecules is to scavenge e$_{aq}^-$ and H$^*$ radicals leading to a production of HO$_2^*/O_2^-$/O$_2^-$ radicals. This couple of radicals was thought to play a role in the oxygen effect by enhancing the toxicity of ionizing radiations in living cells [13-17]. On a chemical point of view, some pathways are suspected to give it a non-negligible role in damage formation since it can serve as the precursor of an oxidative stress. Indeed, stimulated by iron- or copper-ion catalysis, superoxide anions can rapidly react with hydrogen peroxide producing hydroxyl radicals, through the Haber-Weiss reaction:

\[
O_2^- + H_2O_2 \xrightarrow{Fe^{3+}/Fe^{2+}} OH^- + HO^* + O_2
\]  

Moreover, it was suggested that a fast reaction of superoxide with nitrogen monoxide (2), which is synthesized by cells, can produce peroxinitrite ions (ONOO$^-$).

\[
O_2^- + NO' \longrightarrow ONOO^-
\]  

The latter are well known for their deleterious effect on DNA, proteins and lipids. Perhydroxil radical can also efficiently react with nitrogen monoxide, leading to the production of peroxynitrous acid which is also toxic [14]:

\[
HO_2^- + NO' \longrightarrow ONOOH
\]

Perhydroxyl radical is also known to initiate lipid peroxidation [13]. Alternatively, SuperOxide Dismutase (SOD) enzyme was reported to decrease cell radiosensitivity by inducing the dismutation of superoxide anion into hydrogen peroxide [18, 19] according to:

\[
O_2^- + O_2^- \xrightarrow{SOD + 2H^+} O_2 + H_2O_2
\]

The SOD-related decrease of cell radiosensitivity may therefore be due to the decrease in superoxide-anion concentration. Kuninaka et al. [20] reported that a decrease of SOD, which would result in increased quantities of O$_2^-$/ in the mitochondria, augmented apoptosis in cell exposed to radiation [20].

Section 2 gives a brief description of the simulation. Section 3 presents the result obtained first in introducing the various concentrations of oxygen, second in combining the effect of an oxygen concentration and an antioxidant (GSH). An effect of LET will be also presented. Section 4 discusses these results and underlines some correlations between the production of the HO$_2^*/O_2^-$/O$_2^-$ radicals and the oxygen effect.

2. Monte Carlo simulation
The simulation aims at predicting the number and the spatial distribution of free radicals and molecules produced in water by a ionizing radiation. The incident particle could be the $\gamma$-rays of a Cobalt-60 source or a mono-energetic beam of ions. The $\gamma$-rays irradiations were simulated by generating a distribution of electrons for which the energy and the angle were randomly sampled according to the laws of Compton effect, which is the leading process. The irradiations with ions were considered by simulating a segment of ion trajectory. This segment was large enough to get a significant number of chemical species ($2.5 \times 10^5$ species at 1ps), but small enough to assume that the velocity of the ions could be approximated as constant. To reduce statistical fluctuations, the calculated quantities were averaged over several simulated impacts whatever the incident projectile was.
The principle of our simulation is largely inspired by numerous works [21-24]. A detailed description can be found in [25,26]. The simulation was decomposed in three stages: a physical stage, a physicochemical stage and a chemical stage. To simulate the physical stage, the interactions of the ionizing particles with water were described by laws of probability, averaging any effect of water-molecule orientation or local order. The impact of the ionizing particle (Compton electrons or mono-energetic ions) generated a spatial distribution of excited (H\textsubscript{2}O*) or ionic (H\textsubscript{2}O\textsuperscript{+}) water molecules. Ionic species H\textsubscript{2}O\textsuperscript{aq+} could also be created by multiple ionization. All the ejected electrons were followed in the same way. The electron interactions included not only the processes of ionization and excitation but also the processes of elastic diffusion, electron attachment, and phonon creation. The electrons were followed until their energy fell down to a cut-off energy set to 33 meV (energy of thermalization for a temperature of 300K). Geminate recombination process was also simulated. The simulation output was a spatial distribution of low-energy electrons (thermalized electrons), ionized and excited water molecules.

The physicochemical stage describes the medium-relaxation. The thermalized electrons were solvated. The ionized water molecules could either undergo a dissociation process, producing new molecules, or recombine with a thermalized electron to form an excited state. The excited water molecules could also dissociate. When dissociation occurred, the fragments were separated by a specific distance. If multiple-ionization took place, atomic oxygen was produced. The dissociation-channel details and the associated probabilities are available in [26]. In the simulation, all these relaxation channels were sampled by a random process. The outcome of the simulation was a spatial distribution of chemical species. The major species are the hydroxyl radicals, the hydronium ions and the hydrated electrons. These three species mainly issued from single ionization of water molecules according to the channel:

\[
\text{H}_2\text{O} \xrightarrow{\text{radiation}} \text{H}_2\text{O}^+ + e^- \xrightarrow{\text{relaxation}} \text{H}_3\text{O}^+ + \text{HO}^+ + e^-_{\text{aq}}
\]

The chemical stage describes the time evolution of the spatial distribution of the chemical species. Standard chemical models cannot be applied since the spatial distribution can be strongly heterogeneous due in particular to spurs (see figure 1). We therefore developed a Monte Carlo simulation [26], very similar to IRT [21]. This simulation decomposes the whole evolution into a series of short time steps [22-24]. During each time step, the reaction probabilities were calculated for each pair of species as a function of the species diffusion constant, the species charge, the reaction rate, the reaction spin and the reaction radius. A random process selected a part of these reactions according to their reaction probabilities. Since the time step was small, most of the species did not react and simply diffused according to a standard diffusion model. The outcomes of the simulation are the time-dependent radical yields defined as the number of chemical species produced per 100 eV of energy transferred to the sample. Then for a specie I, the yield reads:

\[
Y(\text{specie } i) = 100 \frac{N_i}{\Delta E}
\]

where \(\Delta E\) is energy transferred by the incident particles to the water sample and \(N_i\) is the number of species i issuing from this energy transfer.

As the other simulations of heterogeneous chemistry, our simulation did not include non-independent-pair dynamics effects. It can therefore not describe the time-dependent yields within the femtosecond regime as measured in recent femtolysis (FEMTOsecond radioLYSIS) experiments [27]. Such dynamics may not affect the yield evolution at times larger than few nanoseconds for low-LET radiations. In the domain of extremely high-LET ions, for which the density of species could be very high, an inclusion of non-independent-pair effects should improve the predictions even at time larger than nanoseconds.
Figure 1. Chemical track-segment of a 10 MeV/n carbon ion simulated in water at $10^{-12}$ s (left) and $10^{-6}$ s (right) – Each point represents a chemical species.

We extended the simulation to quantify the effect of a solute on water radiolysis. Since directly introducing the solute to the simulation would dramatically increase the computing time, we applied the approximation introduced by Green et al [28,29]. This approximation consists in representing the distribution of solute as a reservoir. Such an approximation has already been considered by other authors [30-32]. The probability for a species to react with the solute reservoir during the period of time $t$ reads:

$$P(t) = 1 - \exp\left(-\alpha\left(x^2 + y^2\right)\right)$$

with:

$$\alpha = \frac{4\pi D\sigma_{\text{eff}} C_s N_a}{\beta^2}$$

$$x = \beta \sqrt{t}$$

$$y^2 = \frac{v\sigma}{D} \left( \exp(x^2) \text{erfc}(x) - 1 + \frac{2}{\sqrt{\pi}} x \right)$$

$$\beta = \frac{v\sigma}{\sigma_{\text{eff}} \sqrt{D}}$$

where $\sigma$, $v$ and $D$ represent the reaction radius, the reaction velocity and the sum of the diffusion coefficients of both the reactants, respectively. $C_s$ is the concentration of solute and $N_a$ the Avogadro number.

In this work, we have studied the influence of an oxygen concentration on water radiolysis. It is convenient to relate this concentration to the partial pressure of oxygen in air within standard conditions of pressure. We therefore related the concentration and the partial pressure through the Henry laws and considered a Henry constant of $1.3 \times 10^3$ Mol.l$^{-1}$bar$^{-1}$, which corresponds to the
measured value for O_2 solubility in water at 298°K [33]. Hence, for a partial pressure in air of 160 mmHg, the concentration in oxygen is 0.27 mmol/l. Because we did not simulate the effect of pH and in particular the equilibrium of the HO_2^● /O_2^● acid-base couple, we could not in principle distinguish between the relative fractions of both these radicals at large time. In this study we also simulated the effect of a concentration of glutathione (GSH). The following reactions were considered:

\[
\text{OH}^* + \text{GSH} \rightarrow \text{H}_2\text{O} + \text{GS}^* \quad (12)
\]

\[
\text{H}^* + \text{GSH} \rightarrow \text{H}_2 + \text{GS}^* \quad (13)
\]

\[
\text{e}_{aq}^- + \text{GSH} \rightarrow \text{G}^* + \text{HS}^- \quad (14)
\]

These reactions were considered as fully controlled by the diffusion (\(\nu = +\infty\)) and their parameters \(\sigma\) (the reaction radius) were deduced from the reaction constant (see table 1). As a first approximation, we did not introduce in the simulation the products of these reactions. We indeed focus in this paper on the first order effects of GSH on water radiolysis. In a second stage, it would be interesting to include the secondary reactions, which is a challenge since these reactions and their constants are not necessary known. Another approximation was to ignore the direct interaction of the radiations with the solute molecules. Excitation and ionization of the GSH and the oxygen molecules should lead to the formation of other radical species. Considering the concentration of these molecules as compared to the number of available water molecules we expect these contributions to the yield of radicals presented in this work to be low.

Table 1. Constant of the reactions with GSH considered in the simulation

| Reactions                           | \(k\) (L.mol\(^{-1}\).s\(^{-1}\)) | Reference |
|-------------------------------------|----------------------------------|-----------|
| \(\text{HO}^* + \text{GSH} \rightarrow \text{H}_2\text{O} + \text{GS}^*\) | \(9.0\times10^9\)              | 34        |
| \(\text{H}^* + \text{GSH} \rightarrow \text{H}_2 + \text{GS}^*\)          | \(1.0\times10^{10}\)          | 35        |
| \(\text{e}_{aq}^- + \text{GSH} \rightarrow \text{G}^* + \text{HS}^-\)    | \(4.7\times10^{8}\)           | 36        |

3. Results

3.1. Radiolysis of oxygenated water

We simulated the production of radicals induced by the impacts of low-LET protons in water. At 1 picosecond after the impact, the particle mainly produces HO^*, e_{aq}^−, H_3O^+ and in a smaller quantity H^*. Because of their close proximity, these radicals may quickly react with each other, creating molecular species such as H_2 and H_2O_2. Figure 2 clearly shows the decrease of HO^* and e_{aq}^- yields with the time. It also shows results obtained with the same conditions except for the insertion of an oxygen concentration, which corresponds to dissolved oxygen molecules for a normal partial pressure of oxygen in air in standard conditions.

As it is well known, O_2 is an excellent scavenger for solvated electrons and hydrogen radicals. Hence, the most significant changes, with regards to pure water conditions, were marked by a reduction in e_{aq}^- and H^* yields and an increase in HO_2^● and O_2^● yields (see figure 2). The following reactions are involved:
\[ \text{H}^* + \text{O}_2 \longrightarrow \text{HO}_2^*; \ k = 1.27 \times 10^{10} \ \text{L.mol}^{-1}.\text{s}^{-1} \]  

(15)

\[ \text{e}_{\text{aq}}^- + \text{O}_2 \longrightarrow \text{O}_2^*; \ k = 1.84 \times 10^{10} \ \text{L.mol}^{-1}.\text{s}^{-1} \]  

(16)

**Figure 2.** Time-dependant yields of the main species generated by the irradiation with H[65 MeV] of pure water (a) or a partial pressure of oxygen in air of 160 mmHg ([O\(_2\)]=0.27 mMol.L\(^{-1}\)) (b). Species represented are \(\text{e}_{\text{aq}}^-\) (\(\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cd -6 s

*10\(^{-6}\) s

Figure 3.** Variation, in function of the partial pressure of oxygen in air, of the yield in (HO\(_2\)^*+O\(_2\)^*) produced at 1 \(\mu\)s (left) and 10 \(\mu\)s (right) by the \(\gamma\)-rays of a Cobalt-60 source (LET~0.3 keV/\mu m).
One can observe a strong increase in the yield for pressures lower than about 10 to 30 mmHg and a quasi-saturation for pressures larger to this threshold. of the e$_{aq}^-$ and H$^+$ radicals and therefore increased the yield of (HO$_2^+$+O$_2^-$) at 10 microseconds. The saturation pressure corresponded to the minimal pressure value at which most of the e$_{aq}^-$ and H radicals were not scavenged by the oxygen molecules before 10 microseconds. Increasing the pressure fastened the consumption $^+$ radicals were scavenged by the oxygen molecules at 10 microseconds. Figure 3 shows also the same yield calculated at 1 microsecond. For both times, the curves are qualitatively similar. However, the saturation pressure was larger for shorter times: as expected, a larger pressure is required to scavenge most of the e$_{aq}^-$ and H$^+$ radicals a shorter time after the particle impact.

While the time at which the yield is calculated significantly affects the evolution with the pressure, fixing it appears somewhat arbitrary. In particular, in a biological system, there is no reason to consider a specific observation time and the production of (HO$_2^+$+O$_2^-$) has to be considered over a larger time. However this production is expected to be limited by the lifetime of the e$_{aq}^-$ and H$^+$ radicals in the cells.

3.2. Radiolysis of oxygenated water with a concentration of GSH

Anti-oxidants have a major protective role in cells by scavenging the free radicals. The level of antioxidants controls therefore the lifetime of radicals. We expect therefore the production of (HO$_2^+$+O$_2^-$) to be modified by a concentration of antioxidants. We therefore inserted a concentration of GSH into our simulation system. We were particularly interested in GSH because it is known to be one of the most important antioxidants of the cells. Moreover it was reported that the oxygen effect was sensitive to the level of GSH in the cells [10-12]. GSH can be found both in the cell nucleus and cell cytoplasm [37]. We first calculated the time-dependent yields of the main radicals for a concentration of GSH of 1 mmol.L$^{-1}$ and 10 mmol.L$^{-1}$, which correspond to typical concentrations in normal cells [38]. The calculations were performed for a Cobalt-60 source. The main effect is the scavenging of HO$^+$ radicals. However as can be seen in figure 4, the scavenging of e$_{aq}^-$ and H$^+$ radicals is also quite significant at such concentrations and the lifetime of e$_{aq}^-$ and H$^+$ radicals are reduced by GSH to values lower than 10 microseconds. The lifetime of e$_{aq}^-$ radicals is larger than the lifetime of H$^+$ radicals due to a lower reaction constant (see table 1). As a consequence of this scavenging, the production of (HO$_2^+$+O$_2^-$) in oxygenated water by reactions with e$_{aq}^-$ and H$^+$ radicals is achieved before 10 microseconds at such GSH concentrations.

**Figure 4.** – Time-dependant yields of the e$_{aq}^-$ (left) and H$^+$ radicals (right) generated by the γ-rays of a Cobalt-60 source (LET~0.3 keV/μm) in water with a concentration in GSH of 0.1 and 10 mmol.L$^{-1}$. 

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Figure 5 combines the effect of both the dissolved oxygen and the GSH concentration. It represents the yield of \((\text{HO}_2^\bullet + \text{O}_2^\bullet)\) as a function of the partial pressure of oxygen in air. The calculations were performed for a Cobalt-60 source with GSH concentration of 1 mmol.L\(^{-1}\) and at a time large enough as compared to the lifetime of e\(_{aq}\) and H\(^\bullet\) radicals.

![Figure 5](image)

Figure 5. Variation with the partial pressure of oxygen in air of the yields of \((\text{HO}_2^\bullet + \text{O}_2^\bullet)\) produced at 10 \(\mu\)s by the \(\gamma\)-rays of a Cobalt-60 source (LET~0.3 keV/\(\mu\)m) in water with a concentration in GSH of 1 mmol.L\(^{-1}\).

As can be seen on figure 5, the yield of \((\text{HO}_2^\bullet + \text{O}_2^\bullet)\) increased with the pressure and saturated. These features have to be attributed to the competition between the scavenging by the oxygen molecules and the scavenging by the GSH molecules. Below the pressure of saturation, the scavenging by GSH is dominant. Increasing the pressure of oxygen fastened the scavenging by oxygen and increased the production of \((\text{HO}_2^\bullet + \text{O}_2^\bullet)\). The latter production saturated as soon as the scavenging by oxygen got dominant and could capture most of the available e\(_{aq}\) and H\(^\bullet\) radicals.

A last parameter we considered in this study was the effect of the LET. Figure 6 shows the yield of \((\text{HO}_2^\bullet + \text{O}_2^\bullet)\) generated by the impacts of proton and carbon ions in water oxygenated by a partial pressure of 160 mmHg in air and containing a concentration in GSH of 1 mmol.L\(^{-1}\).

![Figure 6](image)

Figure 6. Yield of \((\text{HO}_2^\bullet + \text{O}_2^\bullet)\) expressed as a function of the LET for the \(\gamma\)-rays of a Cobalt-60 source (\(\nabla\)), protons (\(--\triangle--\)) and carbon ions (\(---\circ---\)). The calculations were performed at 10 microseconds for a partial pressure of oxygen of 160 mmHg and a concentration in GSH of 1 mmol.L\(^{-1}\).

We can clearly observe that increasing the LET reduced the production of \((\text{HO}_2^\bullet + \text{O}_2^\bullet)\). The yield fell down to almost zero for the highest LET carbon projectile. This strong reduction arose from the large concentrations of radicals produced in the track (see figure 1). Due to their close proximity, the
radicals interacted with each other before any significant scavenging by the oxygen molecules. The production of \( \text{HO}_2^+ / \text{O}_2^* \) thus became a minor process for high LET.

4. Concluding remarks and perspective

As summarized in the introduction section, superoxide anion \( (\text{O}_2^\cdot) \) and its protonated form, the hydroperoxyl radical \( (\text{HO}_2^\cdot) \) were suspected to play a role in the oxygen effect by enhancing the toxicity of ionizing radiation in living cells [13-17]. The related chemical and biological arguments were a motivation for us to study the parameters that may influence the production of \( \text{HO}_2^\cdot / \text{O}_2^* \). We modified our simulation of the water radiolysis by ionizing particle to insert a concentration of dissolved oxygen and a concentration of antioxidant. As antioxidant we considered glutathione (GSH) since it is one of the most important antioxidants in living cells. Moreover it was reported that the oxygen effect was sensitive to the level of GSH in the cells [10-12]. We have shown that the production of \( \text{HO}_2^\cdot / \text{O}_2^* \) was modified by the presence of GSH. At concentrations ranging from 1 to 10 mmol.L\(^{-1}\) and corresponding to typical concentrations in GSH for normal cells [38], the scavenging of the \( \text{e}_{\text{aq}}^- \) and \( \text{H}^\cdot \) radicals was quite significant. This reduction in the yield of \( \text{e}_{\text{aq}}^- / \text{H}^\cdot \) strongly affected the production of \( \text{HO}_2^\cdot / \text{O}_2^* \), for which the production mainly issued from the reaction of the \( \text{e}_{\text{aq}}^- \) and \( \text{H}^\cdot \) radicals with the oxygen molecules. The competition between the scavenging of \( \text{e}_{\text{aq}}^- \) and \( \text{H}^\cdot \) radicals by the oxygen molecules and by the GSH concentrations led to particular features when plotting the yield of \( (\text{HO}_2^\cdot + \text{O}_2^*) \) as a function of the dissolved oxygen concentration (or equivalently the partial pressure of oxygen in air). At low oxygen pressure, the scavenging by GSH dominated. Increasing the pressure of oxygen fastened the scavenging of the \( \text{e}_{\text{aq}}^- \) and \( \text{H}^\cdot \) radicals by oxygen and led to an increase of the production of \( \text{HO}_2^\cdot / \text{O}_2^* \). We observed however that this increase in the \( \text{HO}_2^\cdot / \text{O}_2^* \) production saturated as soon as the scavenging by oxygen became predominant. For a concentration of 1 mmol.L\(^{-1}\) in GSH, the pressure of saturation amounted to about 20 to 30 mmHg.

From a quantitative point of view, the yield of \( (\text{HO}_2^\cdot + \text{O}_2^*) \) was found as large as 0.3 \( \mu \text{mol.L}^{-1}\) per Gray for a normal pressure of oxygen (160 mmHg) and with a concentration of 1 mmol.L\(^{-1}\) in GSH. The production is therefore several orders of magnitude larger than the standard rate in non-irradiated cells for which the concentration at equilibrium varies between 0.01 mmol.L\(^{-1}\) to 0.1 mmol.L\(^{-1}\) [14, 39]. We also showed that the production of \( \text{HO}_2^\cdot / \text{O}_2^* \) was sensitive to the type of radiation. Increasing the LET of the particle could strongly decrease their production. This reduction was due to the fast reactions between the radicals, which are produced in close proximity for high LET radiation.

Although the comparison between a living cell and a simple system as the one we simulated is limited, we attempt a parallel in the evolution of the production of \( \text{HO}_2^\cdot / \text{O}_2^* \) and the oxygen effect with the studied parameters. A common point is the evolution with the pressure of oxygen. Both increase with the oxygen pressure and saturate. The saturation pressure seems lower for the oxygen effect.

Another common feature of the oxygen effect and the production of \( \text{HO}_2^\cdot / \text{O}_2^* \) is the decrease with the LET and the disappearance at very high-LET. The decrease in the production of \( \text{HO}_2^\cdot / \text{O}_2^* \) is related to the large concentration of energy deposited along the ion trajectory, which leads to efficient reactions between the free radicals and therefore to an inefficient scavenging by the oxygen molecules.

Caution must be exercised in comparing these results because of the complexity of living cells. Many other elements in a cell could indeed modify the production of \( \text{HO}_2^\cdot / \text{O}_2^* \) either by reducing it or by increasing it. Moreover we did not insert in our system the other forms of glutathione (such as GSSG), nor the products of reactions (\( \text{G}^* \), \( \text{HS}^\cdot \), \( \text{GS}^\cdot \)). The latter could also react with oxygen and contribute to the toxicity of the radiations in oxygenated conditions. Moreover, glutathione contributes to cell response to radiations by reducing the damage capacity of \( \text{HO}^\cdot \) radicals. The direct action of the radiations to the cells has also to be considered.

Finally by including the effects of two natural solutes on water radiolysis, this work constitutes one step toward the complexity of radiobiology. Clearly other solutes should be introduced in the
simulation. To this aim a systematic determination of the reaction parameters for the most important solutes will be required. This includes the reactions of the solute with the water radicals but also the cascades of reactions that may follow. Researches, both experimental and theoretical, are still necessary to understand the origin(s) of the oxygen effects. The interest is not only important on a fundamental point of view, but also for applications in Oncology since it could contribute to an improved treatment of hypoxic tumors [40-42].

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