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Research paper

# Radical-induced oxidation of *trans*-resveratrol

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ABSTRACT

*trans*-Resveratrol (RVT) (3,5,4′-trihydroxystilbene), a polyphenolic constituent of red wine, is thought to be beneficial in reducing the incidence of cardiovascular diseases, partly *via* its antioxidant properties. However, the mechanism of action by which *trans*-resveratrol displays its antioxidant effect has not been totally unravelled. This study aimed at establishing a comprehensive scheme of the reaction mechanisms of the direct scavenging of $HO^{\bullet}$ and $O_2^{\bullet-}$ radicals generated by water gamma radiolysis. Aerated aqueous solutions of *trans*-RVT (from 10 to 100 μmol $L^{-1}$) were irradiated with increasing radiation doses (from 25 to 400 Gy) and further analyzed by UV–visible absorption spectrophotometry for detection of *trans*-RVT oxidation products. Separation and quantification of RVT and its four oxidation products previously identified by mass spectrometry, i.e., piceatannol (PCT), 3,5-dihydroxybenzoic acid (3,5-DHBA), 3,5-dihydroxybenzaldehyde (3,5-DHB) and *para*-hydroxybenzaldehyde (PHB), were performed by HPLC/UV–visible spectrophotometry. Determination of the radiolytic yields of *trans*-RVT consumption and oxidation product formation has allowed us to establish balance between *trans*-RVT disappearance and the sum of oxidation products formation. Under our conditions, $O_2^{\bullet-}$ radicals seemed to poorly initiate oxidation of *trans*-RVT, whereas the latter, whatever its initial concentration, quantitatively reacted with $HO^{\bullet}$ radicals, *via* a dismutation mechanism. Two reaction pathways involving $HO^{\bullet}$-induced *trans*-RVT primary radicals have been proposed to explain the formation of the oxidation end-products of *trans*-RVT.

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## 1. Introduction

*trans*-Resveratrol (3,5,4′-trihydroxystilbene) (RVT, Fig. 1) is a polyphenolic phytoalexin which is present in biological materials such as grapes, grape juice, red wine, plums and peanuts [1,2]. This compound has been received much interest for a long time because of its extensive physiological properties. Indeed, several studies have shown its beneficial effects on neurological, hepatic and cardiovascular systems [3,4]. Particularly, RVT has been reported to have atheroprotective properties [5,6]. It has been suggested that the antioxidant properties of RVT are responsible for the protective effect against cardiovascular diseases of consuming moderate amounts of red wine. RVT seems especially able to scavenge hydroxyl radicals produced by a Fenton reaction [7] but as for other polyphenols one has to distinguish between a direct scavenging of free radicals and a chelating effect towards transition metals involved in Fenton reaction [8]. It has also been reported to be a good antioxidant against lipid peroxidation in human blood cells treated by platinum compounds [9], or peroxynitrite [10], in cell membranes [11,12] and low density lipoproteins (LDL) [13–18].

In order to improve the knowledge of RVT antioxidant mode of action, this work focused on its direct antioxidant properties *in vitro* against oxygen-derived free radical species generated in aqueous solution by gamma radiolysis. Gamma radiolysis of water is a well-known method that has many advantages, such as the

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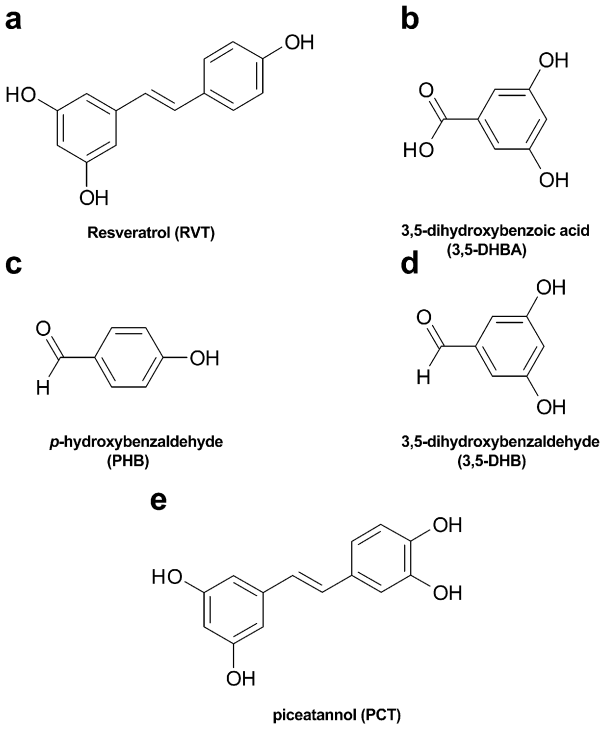



**Fig. 1.** Chemical structure of RVT (a) and of its oxidation products generated by free radicals attacks [19], i.e., 3,5-dihydroxybenzoic acid (b), *p*-hydroxybenzaldehyde (c), 3,5-dihydroxybenzaldehyde (d) and piceatannol (e).

homogeneous production of known quantities of free radicals (such as superoxide anion $O_2^{\bullet-}$ or hydroxyl radical $HO^{\bullet}$), as well as the possibility to selectively produce one specific radical to be studied at a time. Oxygen-derived radicals thus generated have been used to initiate one-electron oxidation of RVT dissolved in water. In a previous work, we have identified by mass spectrometry the oxidation end-products of RVT resulting from the direct attack of $HO^{\bullet}/O_2^{\bullet-}$, i.e., 3,5-dihydroxybenzoic acid (3,5-DHBA), 3,5-dihydroxybenzaldehyde (3,5-DHB), *para*-hydroxybenzaldehyde (PHB) and piceatannol (*trans*-3,5,3′,4′-tetrahydroxystilbene, PCT) (Fig. 1) [19]. However, only one radiation dose (400 Gy) and only one concentration of RVT (100 μmol $L^{-1}$) were studied. In order to specify the $HO^{\bullet}/O_2^{\bullet-}$-induced oxidation mechanism of RVT, the present paper focuses on the effect of increasing radiation doses (from 25 to 400 Gy) on aqueous RVT solutions at five concentrations (from 10 to 100 μmol $L^{-1}$), in order to establish a comprehensive reaction mechanism of RVT oxidation by $HO^{\bullet}/O_2^{\bullet-}$ radical species.

## 2. Materials and methods

### 2.1. Chemicals and reagents

*trans*-Resveratrol (RVT, $M = 228$ g $mol^{-1}$) was purchased from Cayman Chemical Company (Spi-Bio, Montigny-le-Bretonneux, France). RVT solutions (10, 20, 30, 50, 70 and 100 μmol $L^{-1}$) were prepared by sonication for 2 h in 10 mmol $L^{-1}$ phosphate buffer at pH 7 in ultra-pure water (Maxima Ultra-Pure Water, ELGA, resistivity 18.2 MΩ). Aqueous solutions of RVT were kept in dark to avoid isomerization into *cis*-RVT. Before irradiation, they were systematically checked by UV–visible spectrophotometry (according to a previously developed method [20]) to make sure that no isomerization into *cis*-RVT has occurred. Authentic piceatannol was purchased from ABCys (Paris, France), and 3,5-DHBA, 3,5-DHB and PHB from Sigma–Aldrich (St-Louis, MO, USA).

### 2.2. Gamma radiolysis

Radiolysis corresponds to the chemical transformations of a solvent due to the absorption of ionizing radiations, which allows, within a few nanoseconds, the production of a homogeneous solution of reactive oxygen species. In addition, this method allows selective generation of specific radicals from the solvent, so that it is possible to study their action towards the dissolved compounds. Radiolytically generated radicals are independent of the nature and of the concentration of the dissolved compound as long as its concentration remains lower than or equal to $10^{-2}$ mol $L^{-1}$ [21].

Gamma radiolysis was carried out by using an IBL 637 irradiator (CIS Biointernational, Gif-sur-Yvette, France) of $^{137}Cs$ source, whose activity was ≈ 222 TBq (6000 Ci). In our experiments the dose rate was 10 Gy $min^{-1}$. The dosimetry was determined by the Fricke method [22], namely radio-oxidation of a 1 mmol $L^{-1}$ of iron(II) sulphate solution in 0.4 mol $L^{-1}$ sulphuric acid, taking $\lambda_{max}(Fe^{3+}) = 304$ nm, $\varepsilon_{(304\ nm)} = 2204$ L $mol^{-1}$ $cm^{-1}$ and a radiolytic yield of $G(Fe^{3+}) = 16.2 \times 10^{-7}$ mol $J^{-1}$ at 25 °C. Different radiation doses, ranging from 25 to 400 Gy, were delivered to 1.5 mL of solution depending on the time of the exposure to the γ-ray source: the longer the time, the higher the radiation dose. For each experimental set, 1.5 mL of non-irradiated solution was taken as a control. Prior to each set of experiments, glassware was carefully washed with TFD4 soap (Franklab, France), rinsed with ultra-pure water (resistivity 18.2 MΩ) and finally heated at 400 °C for 4 h to avoid any pollution by organic compounds.

Water radiolysis by γ-rays generates radical and molecular species (i.e., $e^-_{aq}$, $HO^{\bullet}$, $H^{\bullet}$, $H_2$ and $H_2O_2$). Under aerated conditions – dissolved molecular oxygen concentration around $10^{-4}$ mol $L^{-1}$ – hydroxyl and superoxide radicals (this latter resulting from the scavenging of $e^-_{aq}$ and $H^{\bullet}$ species by $O_2$) are simultaneously produced with respective radiolytic yields (*G*-values expressed in mol $J^{-1}$) of $2.8 \times 10^{-7}$ and $3.4 \times 10^{-7}$ mol $J^{-1}$ [21]. To selectively obtain superoxide anion, 0.1 mol $L^{-1}$ sodium formate was added into aqueous solutions in order to convert all radical species ($e^-_{aq}$, $HO^{\bullet}$, $H^{\bullet}$) into $O_2^{\bullet-}$ radicals with a final *G*-value of $6.2 \times 10^{-7}$ mol $J^{-1}$ [21].

### 2.3. Analysis

Detection of the formation of RVT oxidation products was achieved by spectrophotometric measurements with an UV–visible spectrophotometer (Beckman DU 800, Villepinte, France) at room temperature, with a 0.2-cm or 1-cm pathlength quartz cell. Samples were scanned from 190 to 500 nm. RVT in phosphate-buffered (10 mmol $L^{-1}$, pH 7) aqueous solutions had a maximum absorption at 304 nm ($\varepsilon_{304} = 30\ 135$ L $mol^{-1}$ $cm^{-1}$ [20]) and Beer–Lambert law was applicable within the studied range of concentration (10–100 μmol $L^{-1}$).

Separation of RVT from its oxidation end-products was performed by high pressure liquid chromatography (HPLC) (P1000 XR, Thermo Separation Products, Les Ulis, France) coupled to a UV–visible spectrophotometer (UV 3000, Thermo Separation Products, Les Ulis, France). Chromatographic separation was performed using a reverse-phase C18 analytical column (250 mm × 4.6 mm, 5 μm, Kromasil, A.I.T, Houilles, France). Gradient elution was carried out with 0.5% (v/v) acetic acid in $H_2O$ (mobile phase A) and 0.5% (v/v) acetic acid in acetonitrile (mobile phase B) at a flow rate of 1.0 mL $min^{-1}$. The mobile phase was degassed ultrasonically before use. The gradient was programmed with the following time course: 20% mobile phase B at 0 min, linear increase to 80% mobile phase B

at 30 min, 20% mobile phase B at 31 min and held 5 min. A volume of 100 μL of solution was injected into the column, maintained at 30 °C. Concentrations of RVT and its oxidation end-products (PCT, 3,5-DHBA, 3,5-DHB and PHB) were determined from the HPLC peak area. Calibration curves were built with standard solutions of authentic PCT, 3,5-DHBA, 3,5-DHB and PHB (0–100 μmol $L^{-1}$, phosphate buffer 10 mmol $L^{-1}$, pH 7) by detection at 304 nm. Linear regression was obtained with coefficient of determination $r^2 > 0.99$: $y = 153\ 793 \times x$ with $r^2 = 0.996$ for RVT, $y = 92\ 800 \times x$ with $r^2 = 0.996$ for PCT, $y = 12\ 414 \times x$ with $r^2 = 0.992$ for 3,5-DHBA, $y = 11\ 400 \times x$ with $r^2 = 0.993$ for 3,5-DHB and $y = 19\ 592 \times x$ with $r^2 = 0.993$ for PHB. All experiments were performed in triplicate, and experimental data are expressed as means ± standard deviation (SD).

## 3. Results

Before studying the direct attack of $HO^{\bullet}/O_2^{\bullet-}$ towards RVT, the scavenging capacities of phosphate buffer (PB) and radiolytically generated $H_2O_2$ ($G = 0.7 \times 10^{-7}$ mol $J^{-1}$) towards free radicals were assessed. At pH 7, $H_2PO_4^-$ and $HPO_4^{2-}$ might react with the hydroxyl radicals ($k \approx 10^6$ L $mol^{-1}$ $s^{-1}$ [23]), and $H_2O_2$ might also be oxidized by $HO^{\bullet}$ radicals ($k \approx 10^7$ L $mol^{-1}$ $s^{-1}$ [24]) thus resulting in misinterpretation of the experimental results. Aqueous 10 μmol $L^{-1}$ solutions of RVT in the presence of absence of either 0.1, 1 and 10 mmol $L^{-1}$ phosphate buffer, or of 0.05, 0.5 and 5 mmol $L^{-1}$ $H_2O_2$, were irradiated and analyzed by absorption spectrophotometry at 304 nm. The oxidation of RVT was not found to be influenced by the presence of phosphate buffer or $H_2O_2$ (Supplementary material, Fig. s1), even at the highest concentration of the latter compounds.

Phosphate buffered (10 mmol $L^{-1}$, pH 7) aqueous solutions of RVT 100 μmol $L^{-1}$ were irradiated at increasing radiation doses from 25 to 400 Gy (+control, 0 Gy) and absorbance was measured between 190 and 490 nm. As RVT was oxidized, the absorption at 304 nm decreased (Fig. 2a), thus illustrating the oxidation of RVT. At the same time, two new spectral bands at 252 and 360 nm increased with the radiation dose, and can be attributed to the oxidation products. Among them, the former one was previously attributed to the isomerization of *trans*-RVT into *cis*-RVT ($\varepsilon_{cis(252\ nm)} = 6557$ L $mol^{-1}$ $cm^{-1}$ [20]). These results are confirmed by the differential absorption spectra (Fig. 2b).

The quantification of the disappearance of RVT and the formation of the four oxidation products was conducted by HPLC analysis. A chromatogram of RVT 50 μmol $L^{-1}$ before and after irradiation (400 Gy) is presented in Fig. 3. Only RVT was detected in the control ($t_r = 13.9$ min), whereas at 400 Gy, 3,5-DHBA, 3,5-DHB, PHB and PCT were respectively detected at $t_r = 3.9$ min, $t_r = 6.6$ min, $t_r = 7.9$ min and 12.1 min by comparison with previous mass spectrometric analyses and with retention times of standard solutions [19]. The concentration of RVT and its oxidation end-products was measured and reported as a function of the radiation dose for an initial concentration of 50 μmol $L^{-1}$ RVT (Fig. 4). The concentration of RVT decreased, thus confirming the oxidation of RVT as a function of the radiation dose (Fig. 4a), whereas the concentration of 3,5-DHBA, 3,5-DHB, PHB and PCT increased with the radiation dose, indicating the simultaneous formation of these four oxidation products (Fig. 4b). The same result was obtained whatever the initial concentration of RVT (10, 20, 30, 70 and 100 μmol $L^{-1}$) (Supplementary material, Fig. s2). The radiolytic yields ($G$) of RVT consumption and oxidation product formation – determined as the slope of the initial tangent of each curve – are reported in Table 1. Whatever its initial concentration, the radiolytic yield of RVT consumption remains constant, with an average value of $G_{(-RVT)} = (1.43 \pm 0.08) \times 10^{-7}$ mol $J^{-1}$. All hydroxyl radicals ($HO^{\bullet}$) generated during gamma radiolysis are scavenged by RVT, and

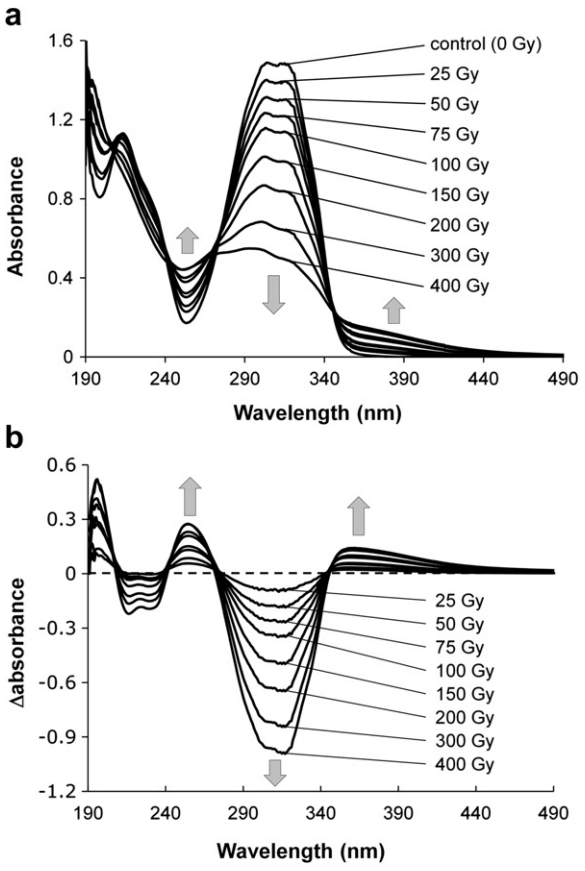



**Fig. 2.** Spectral characterization of RVT oxidation by $HO^{\bullet}/O_2^{\bullet-}$. Absorption spectra of phosphate buffered (10 mmol $L^{-1}$, pH 7) aerated aqueous solution of 100 μmol $L^{-1}$ RVT irradiated at 25, 50, 75, 100, 150, 200, 300 and 400 Gy. (a) Absolute absorption spectra (reference: phosphate buffer, 10 mmol $L^{-1}$, pH 7), (b) differential absorption spectra (reference: non-irradiated RVT solutions). Optical pathlength: l = 1 cm, dose rate: I = 10 Gy $min^{-1}$. The arrows indicate the evolution of the absorption bands.

because $G(-RVT) = ½G_{HO^{\bullet}} \times (G_{HO^{\bullet}} = 2.8 \times 10^{-7}$ mol $J^{-1}$ [21]), radio-induced oxidation of RVT appears as proceeding *via* a dismutation reaction (equation (1)).

$$2RVT \xrightarrow{HO^{\bullet}} \text{primary radicals} \rightarrow \rightarrow RVT + \text{oxidation products} \qquad (1)$$

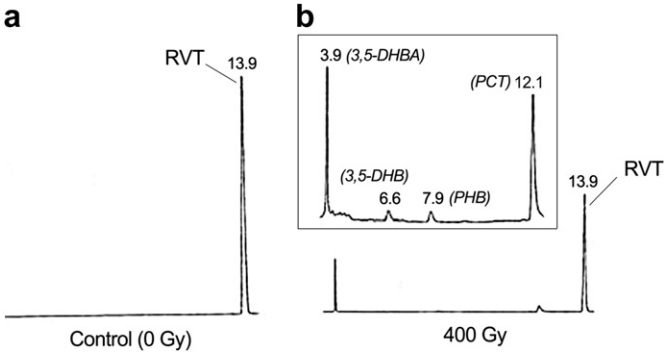



**Fig. 3.** HPLC chromatograms of aerated aqueous solution of resveratrol (a) non-irradiated and (b) irradiated at 400 Gy (action of $HO^{\bullet}/O_2^{\bullet-}$ radicals), with UV detection at 304 nm; 50 μmol $L^{-1}$ initial RVT concentration, phosphate buffered (10 mmol $L^{-1}$) aqueous aerated solution at pH 7 (dose rate I = 10 Gy $min^{-1}$). RVT = *trans*-resveratrol; 3,5-DHBA = 3,5-dihydroxybenzoic acid; 3,5-DHB = 3,5-dihydroxybenzaldehyde; PHB = *para*-hydroxybenzaldehyde; PCT = piceatannol.

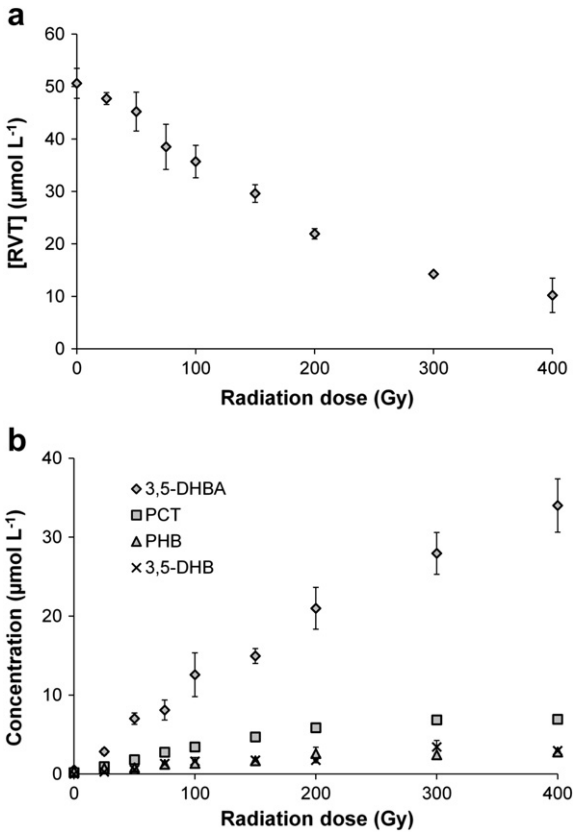



**Fig. 4.** Consumption of resveratrol and production of radical-induced oxidation products (action of $HO^{\bullet}/O_2^{\bullet-}$ radicals). Remaining non-oxidized resveratrol (a) and generated 3,5-DHBA, PCT, PHB and 3,5-DHB (b) as a function of the radiation dose (dose rate $I = 10\ Gy\ min^{-1}$); 50 µmol $L^{-1}$ aqueous aerated solutions of resveratrol, phosphate buffered (10 mmol $L^{-1}$), pH 7, irradiated at 25, 50, 75, 100, 150, 200, 300 and 400 Gy (+control, 0 Gy). Results are expressed as mean ± SD ($n = 3$).

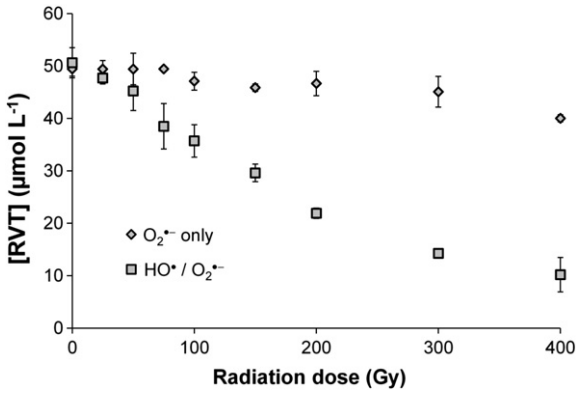



**Fig. 5.** Remaining non-oxidized RVT as a function of the radiation dose (dose rate $I = 10\ Gy\ min^{-1}$). Aqueous aerated solutions (phosphate buffered, 10 mmol $L^{-1}$, pH 7) of RVT 50 µmol $L^{-1}$ without (simultaneous production of $HO^{\bullet}$ and $O_2^{\bullet-}$ radicals) or with sodium formate 0.1 mol $L^{-1}$ (specific production of $O_2^{\bullet-}$ radicals), irradiated at 25, 50, 75, 100, 150, 200, 300 and 400 Gy (+control, 0 Gy). Results expressed as mean ± SD ($n = 3$).

Among the oxidation products generated, 3,5-DHBA exhibits the highest radiolytic yield of formation and has been previously identified as an *in vivo* metabolite of RVT resulting from a bioconversion by cytochrome P450 [25,26]. This result is supported by the study of Makris and Rossiter [27], showing that the major products from quercetin radical-induced oxidation was protocatechuic acid (3,4-dihydroxybenzoic acid) resulting from an oxidative fragmentation of quercetin. Two other minor products, 3,5-DHB and PHB, were detected and accounted for 8% and 7% respectively (Table 1).

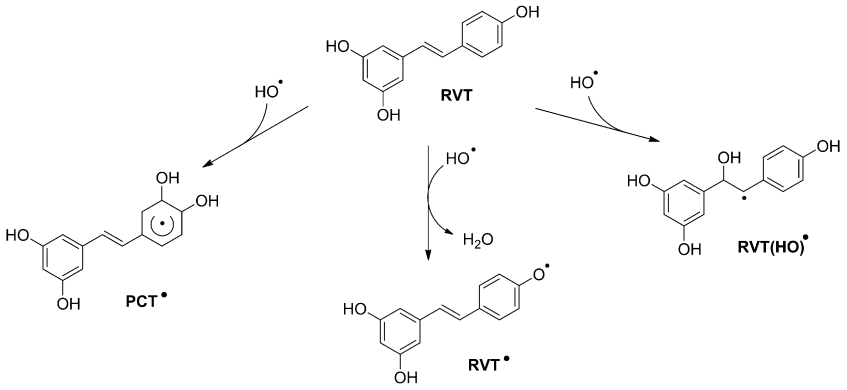
Equation (1) implies that the effect of $O_2^{\bullet-}$ radicals on the initiation of RVT oxidation is supposed to be very weak. To check for this, RVT aqueous solutions at concentration ranging from 10 to 100 µmol $L^{-1}$ were irradiated in the presence of sodium formate (0.1 mol $L^{-1}$). Under these experimental conditions, $O_2^{\bullet-}$ radical is the only radical species produced by water radiolysis with a formation yield of $6.2 \times 10^{-7}$ mol $J^{-1}$ (see Materials and methods). The concentration of remaining non-oxidized RVT has been reported as a function of the radiation dose for an aqueous solution of RVT 50 µmol $L^{-1}$, oxidized either by $HO^{\bullet}/O_2^{\bullet-}$ or by $O_2^{\bullet-}$ radicals alone (Fig. 5). The consumption of RVT is much less important when $O_2^{\bullet-}$ is the only species able to initiate RVT oxidation. At 300 Gy, 72% of RVT was consumed by $HO^{\bullet}/O_2^{\bullet-}$ vs. a few 10% by $O_2^{\bullet-}$ alone, what would represent about 5% in the conditions were $HO^{\bullet}$ and $O_2^{\bullet-}$ are produced together $(G(O_2^{\bullet-})_{HO^{\bullet}/O_2^{\bullet-}} = 3.4 \times 10^{-7}\ mol\ J^{-1}$ and $G(O_2^{\bullet-})_{O_2^{\bullet-}\ alone} = 6.2 \times 10^{-7}\ mol\ J^{-1}$, thus $10\% \times (3.4/6.2) = 5.5\%$). However, the radiolytic yield of RVT consumption − the slope of the initial tangent to the curve − remains close to zero when $O_2^{\bullet-}$ is specifically generated. Under our experimental conditions, the effect of $O_2^{\bullet-}$ radicals can be considered as negligible regarding the initiation of RVT oxidation, thus implying that superoxide radicals would mainly dismutate in such conditions ($O_2^{\bullet-} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2$, $k = 6 \times 10^5\ L\ mol^{-1}\ s^{-1}$ at pH 7, [28]). This result is in good accordance with Bader et al. [29] that showed that only hydroxyl radicals were responsible for RVT oxidation in similar experimental conditions.

## 4. Discussion

According to our experimental results, neither phosphate buffer anions nor hydrogen peroxide compete with RVT towards the action of $HO^{\bullet}/O_2^{\bullet-}$ radicals. Moreover, it seems that superoxide radicals are poorly able to initiate RVT oxidation, which is in agreement with a study of Jia et al. showing by EPR that the direct

**Table 1**
Radiolytic yields $G$ ($10^{-7}$ mol $J^{-1}$, mean (standard deviation)) of disappearance of RVT submitted to the action $HO^{\bullet}/O_2^{\bullet-}$ radicals, and radiolytic yields of production of the oxidation products (along with their sum, $\Sigma P_{ox}$). Initial concentrations of RVT were 10, 20, 30, 50, 70 and 100 µmol $L^{-1}$. Radiolytic yields are given by the slopes of initial tangents of the curves in Fig. 4 and Fig. s2. Average (av.) values (standard deviation) of radiolytic yields of RVT consumption and oxidation product formation ($\sum P_{ox}$) are indicated. RVT = *trans*-resveratrol; 3,5-DHBA = 3,5-dihydroxybenzoic acid; 3,5-DHB = 3,5-dihydroxybenzaldehyde; PHB = *para*-hydroxybenzaldehyde; PCT = piceatannol.

| | Initial RVT concentration | | | | | | Av. |
|---|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 50 | 70 | 100 | |
| RVT | 1.39 (0.07) | 1.32 (0.02) | 1.55 (0.03) | 1.42 (0.02) | 1.50 (0.02) | 1.41 (0.02) | 1.43 (0.08) |
| 3,5-DHBA | 0.75 (0.08) | 0.92 (0.09) | 0.86 (0.09) | 0.93 (0.09) | 1.00 (0.09) | 0.96 (0.09) | 0.90 (0.09) |
| 3,5-DHB | 0.10 (0.01) | 0.12 (0.01) | 0.11 (0.01) | 0.13 (0.01) | 0.13 (0.01) | 0.13 (0.01) | 0.12 (0.01) |
| PHB | 0.09 (0.01) | 0.09 (0.01) | 0.09 (0.01) | 0.12 (0.01) | 0.10 (0.01) | 0.11 (0.01) | 0.10 (0.01) |
| PCT | 0.26 (0.03) | 0.30 (0.03) | 0.29 (0.03) | 0.30 (0.01) | 0.31 (0.03) | 0.32 (0.03) | 0.30 (0.02) |
| $\sum P_{ox}$ | 1.2 (0.13) | 1.43 (0.14) | 1.35 (0.14) | 1.48 (0.12) | 1.54 (0.14) | 1.52 (0.14) | 1.42 (0.13) |



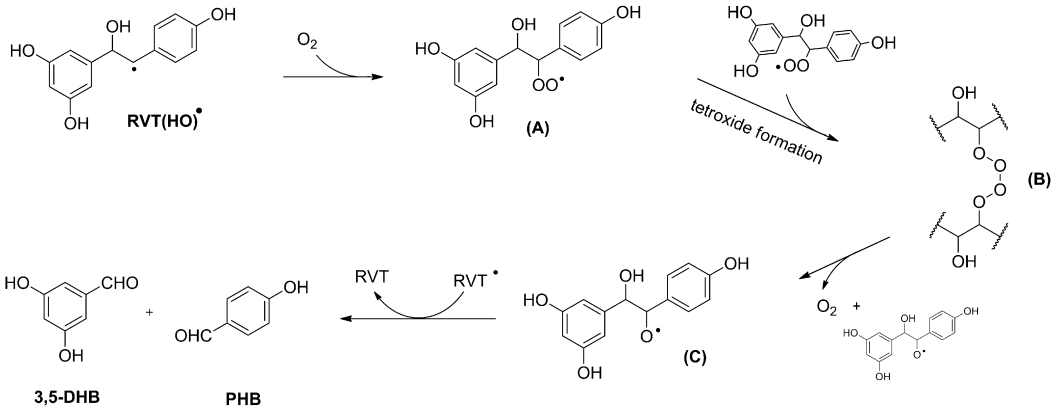

**Scheme 1.** Proposed mechanism of primary RVT radical generation.

attack of superoxide radicals does not induce RVT oxidation for a concentration range from 10 to 100 μmol $L^{-1}$ [30]. Thus, the hydroxyl radical is the only species initiating RVT oxidation.

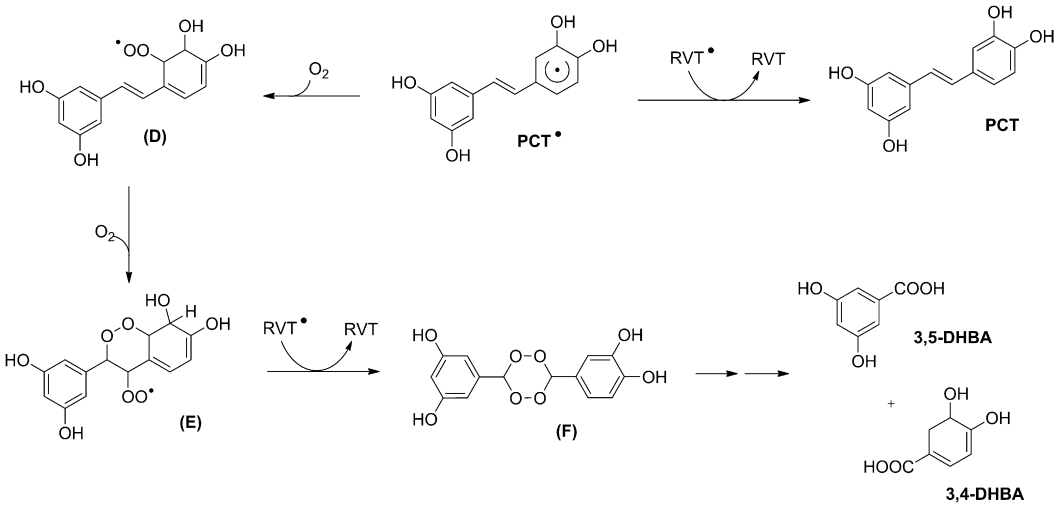
A reaction mechanism can be proposed for the generation of the primary radical, resulting from a single attack of one hydroxyl radical towards one RVT molecule (Scheme 1). The first radical – noted RVT• – is generated by the abstraction of hydrogen located on the hydroxyl group in position 4′ of the phenol moiety of RVT. Bond dissociation energy (BDE) is known to be small for phenol and, in addition, the radical thus formed is close to a semiquinone resonance structure with the most extensive resonance system (comparing to 3- and 5-radical, on the resorcinol moiety of RVT) [31]. The formation of this radical has been previously observed by Stojanovic et al. [32] by pulse radiolysis experiments of a system involving RVT and trichloromethylperoxyl radicals. It has also been proposed as one of the first step of primary radical formation during reaction between RVT and galvinoxyl radicals [33]. The second main pathway for primary radical generation corresponds to the addition of HO•, which could occur itself *via* two ways: first, HO• radical could add to the phenol moiety of RVT, in position 3′ (ortho) because of the π-donor effect of the hydroxyl group in position 4′ [34], leading to a piceatannol-like radical PCT•; second, hydroxyl radical could add to the aliphatic double bond of RVT, leading to RVT(HO)• for which the carbon-centred radical is stabilized by the π-donor effect of the hydroxyl group in position 4′, extending the resonance system to the whole phenol moiety. The latter radical has been previously proposed in similar experimental conditions [29].

Starting with these three primary radicals, the formation of the oxidation end-products of RVT would proceed according to two main pathways. First, because of the strong stabilization of the radical, RVT• is assumed not to react with molecular oxygen (addition) but with other radical transient species (biradical reactions). Among the two other primary radicals, RVT(HO)• would undergo the rapid addition of molecular oxygen (Scheme 2), to form the peroxyl radical (A). The condensation of two of them leads to the tetroxide dimer (B) that could lose one molecular oxygen to form the alkoxyl radical (C). A biradical reaction between this latter and RVT•, proceeding by the hydrogen abstraction on the aliphatic hydroxyl group, finally leads to 3,5-dihydroxybenzaldehyde (3,5-DHB) and *para*-hydroxybenzaldehyde (PHB), along with the regeneration of one molecule of RVT. The simultaneous formation of 3,5-DHB and PHB by a single reaction pathway is consistent with our experimental results because the formation of 3,5-DHB and PHB represents 7-8% of all the oxidation products, for each (Table 1). Thus, implicitly, the formation of RVT(HO)• would appear as a non-preferential way in the radical-induced oxidation of RVT.

The second way of the primary radical evolution starts with PCT• (Scheme 3). A biradical reaction with RVT• leads to the formation of piceatannol by re-aromatization and regenerates one molecule of resveratrol. This first reaction competes with the rapid addition of molecular oxygen, to form the peroxyl radical (D), followed by a cyclization and a second addition of molecular oxygen to finally lead to the endoperoxoperoxyl radical (E). Re-aromatization could then proceed by hydrogen abstraction in position 3′ and rearrangement, producing the cyclic non-radical di-endoperoxide (F). The latter could chemically evolve to finally lead to 3,5-dihydroxybenzoic acid (3,5-DHBA) and 3,4-dihydroxybenzoic acid (3,4-DHBA, or protocatechuic acid). The two latter isomers were




**Scheme 2.** Proposed mechanism of RVT(HO)• radical secondary reaction, leading to oxidation end-products.




**Scheme 3.** Proposed mechanism of PCT• radical secondary reaction, leading to oxidation end-products.

probably co-eluted in our experimental conditions – i.e. protocol based on reversed-phase chromatographic separation – that is the reason why only one peak was detected (Fig. 3). The way of PCT• evolution would appear as the main reaction way of RVT oxidation since the three products formed – i.e., PCT, 3,4-DHBA and 3,5-DHBA – account for 85% of the total amount of the oxidation products (Table 1). Moreover, the addition of molecular oxygen on PCT• is likely faster than the biradical reaction between PCT• and RVT• since 3,4- and 3,5-DHBA are preferentially formed.

## 5. Conclusion

The aim of this study was to propose a comprehensive scheme of the reaction mechanisms of the direct scavenging of HO• and $O_2^{\bullet-}$ radicals generated by water gamma radiolysis, thanks to a quantitative analysis (determination of the radiolytic yields) of the disappearance of *trans*-RVT and of the concomitant formation of *trans*-RVT-derived oxidation products previously identified by mass spectrometry. Whereas $O_2^{\bullet-}$ radicals seemed to poorly initiate oxidation of *trans*-RVT, HO• radicals quantitatively reacted with *trans*-RVT, *via* a dismutation mechanism. Two reaction pathways involving HO•-induced *trans*-RVT primary radicals have been proposed to explain the formation of the oxidation end-products of *trans*-RVT. We have shown that piceatannol (PCT) and 3,5-dihydroxybenzoic acid (3,5-DHBA) accounted for ~85% of the oxidized *trans*-RVT, whereas 3,5-dihydroxybenzaldehyde (3,5-DHB) and *para*-hydroxybenzaldehyde (PHB) accounted for ~15%. These results help understand the direct radical scavenging of *trans*-RVT, property involved in the beneficial antioxidant effect of this natural polyphenol.

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## Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biochi.2011.11.005

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