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Effect of oxygen on the hydroxylation of adenine by photolytically generated hydroxyl radical

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Abstract

The adduct formed in aqueous solution after the reaction of photolytically generated $HO^{\bullet}$ radical with adenine at the C(8) position, Ade–8-OH$^{\bullet}$, is oxidized by ferricyanide or molecular oxygen to give 8-hydroxyadenine (8-OH–Ade). In the former case, Ade–8-OH$^{\bullet}$ is oxidized by electron transfer, whereas, in the latter, it undergoes oxygen addition, forming a peroxyl radical, Ade–8-OH–$O_2^{\bullet}$. From a comparison of the yields of 8-OH–Ade in both cases, it is found that only 34%, at most, of the Ade–8-OH–$O_2^{\bullet}$ radicals eliminate superoxide anion. © 1997 Elsevier Science S.A.

Keywords: Adenine; Radical redox reaction; Hydroxyl radical; Molecular oxygen

1. Introduction

A significant fraction of the biological lesions undergone by living tissues after exposure to ionizing radiation result from the reaction of the hydroxyl radical $HO^{\bullet}$, produced by water radiolysis, with DNA [1]. Of the constituents of this molecule, the purine and pyrimidine bases are particularly sensitive, since their π bonds are a preferential target for $HO^{\bullet}$. As far as the purine bases are concerned, some frequently observed chemical changes after this reaction are the formation of 5-formamidopyrimidines and 8-hydroxypurines [2–4]. Both products have the hydroxyl adduct at C(8) (Ade–8-OH$^{\bullet}$) as their precursor. The reducing properties of this adduct have been demonstrated by showing that the formation of formamidopyrimidines, which requires the imidazole ring opening of Ade–8-OH$^{\bullet}$, is strongly inhibited by oxygen [4]. The presence of oxygen has also been reported to lead to an increase in the yield of 8-hydroxypurines [5,6]. However, at least in the case of adenine, the efficiency of molecular oxygen in promoting the oxidation of Ade–8-OH$^{\bullet}$ to 8-hydroxyadenine (8-OH–Ade) is far from complete. Previous studies have shown that the yields of 8-OH–Ade after γ radiolysis of aqueous solutions of adenine are increased more by ferricyanide, which is a commonly used oxidant for cyclohexadienyl-type radicals [7], than by oxygen [6]. In this work, the $HO^{\bullet}$ radical was generated by UV photolysis of H_2O_2, and its reaction with adenine was followed by observing the formation of 8-OH–Ade. By comparing the yields of this product using ferricyanide or oxygen as oxidant for Ade–8-OH$^{\bullet}$, conclusions can be drawn concerning the mechanisms involved in each case, namely the extent of superoxide elimination from Ade–8-OH–$O_2^{\bullet}$, which is required for the formation of 8-OH–Ade.

2. Experimental details

2.1. Chemicals

Adenine (p.a., Sigma), KH_2PO_4, Na_2HPO_4 and potassium ferricyanide (p.a., Merck) were used without further purification. Hydrogen peroxide (30% v/v aqueous solution, p.a., Panreac) was titrated with $KMnO_4$ in acidic medium and diluted to 0.1 M before use. 8-Hydroxyadenine was synthesized by the method of Cavalieri and Bendich [8].

2.2. Apparatus

UV photolysis was carried out using a Hanau 150 W high-pressure Hg lamp and 2.5 ml quartz cells placed 5 cm from the lamp. Analysis of the irradiated solutions was performed by a high performance liquid chromatography (HPLC) system consisting of a Shimadzu LC-10AS pump, a Reodhyne model 5125 injector fitted with a 20 µl loop, a reversed phase

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(octadecylsilane) Merck analytical column and a PARC EG&G model 400 electrochemical detector at +650 mV vs. the normal hydrogen electrode (NHE). Chromatographic data were acquired by a Merck Hitachi D-2500 integrator. The eluent was a 10% (v/v) MeOH aqueous solution, buffered at pH 7 with both 1 mM KH_2PO_4 and Na_2HPO_4.

2.3. Procedure

The solutions to be irradiated were prepared immediately before use and contained 0.5 mM adenine and 1 mM H_2O_2. The pH was adjusted to pH 7 with both 5 mM KH_2PO_4 and Na_2HPO_4. When required, oxygen was removed from the solution by bubbling with argon. After irradiation for periods in the range 5–20 min, the solutions were immediately analysed by HPLC.

3. Results and discussion

Electrochemical detection has been successfully employed for the analysis of 8-hydroxypurines formed by the radiolytically or chemically induced hydroxylation of purines [9,10]. This detection method is particularly adequate for the analysis of 8-OH–Ade, since the retention times of adenine and 8-OH–Ade are similar under the conditions described above, and adenine is electrochemically inactive at +650 mV.

After UV irradiation, HPLC electrochemical analysis of the solutions reveals the presence of 8-OH–Ade, even in the absence of oxygen (Fig. 1). No changes in the composition of the solutions are observed on irradiation without H_2O_2, or with the addition of 0.1 M ethanol, as an $HO^\bullet$ scavenger, before irradiation. These observations rule out the formation of 8-OH–Ade by photochemical reactions induced by UV light absorption by adenine. The formation of 8-OH–Ade has previously been suggested to occur by the oxidation of Ade–8-OH$^\bullet$ by other adenine radicals or added oxidants [6,11]. However, we observe that the yields of 8-OH–Ade decrease in the presence of oxygen (Fig. 2). This result strongly suggests that molecular oxygen, in spite of reacting very rapidly with Ade–8-OH$^\bullet$ [6], is relatively ineffective in promoting the oxidation of this adduct to 8-OH–Ade. By removing oxygen from the solutions and using ferricyanide anion as an oxidant for Ade–8-OH$^\bullet$, the concentration of 8-OH–Ade formed after a 10 min photolysis period is significantly increased, tending towards a limiting plateau with increasing ferricyanide concentration (Fig. 3, for deaerated solution). It should be noted that the quantum yield of photolysis of H_2O_2 into $HO^\bullet$ radicals is independent of the pH and the presence of oxygen [12]. The ferricyanide anion is a good oxidant for cyclohexadienyl-type radicals, reacting typically by electron transfer [7]; the rate constant of reaction with Ade–8-OH$^\bullet$ is $6\times10^9\ M^{-1}\ s^{-1}$ [6]. Therefore the lower yields of 8-OH–Ade, obtained in the presence of oxygen, must be due to the fact that this molecule reacts with organic radicals preferentially by addition and not by outer-sphere electron transfer. In the case of the Ade–8-OH$^\bullet$ adduct, the peroxyl radical obtained after oxygen addition can only lead to the formation of 8-OH–Ade after elimination of superoxide anion and deprotonation. The concentration of 8-OH–Ade, formed after a certain period of photolysis t, is given by Eq. (1), which can be deduced from the following equations by applying the steady state approximation for $HO^\bullet$ and Ade–8-OH$^\bullet$ (k_{bim} from [13]; k_3 from [6])

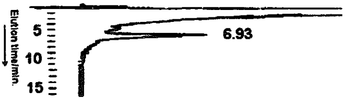


Fig. 1. Electrochemical chromatogram (for conditions, see Section 2) of a solution containing initially 0.5 mM adenine and 1 mM H_2O_2 at pH 7 under argon after 10 min of UV irradiation. The first broad peak is due to H_2O_2 and other unretained solutes; that at 6.93 min is due to 8-OH–Ade.

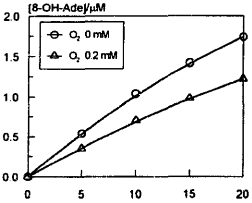


Fig. 2. Formation of 8-OH–Ade by the reaction of 0.5 mM adenine with $HO^\bullet$, generated by the UV photolysis of 1.0 mM H_2O_2, in aqueous solution at pH 7 (aerated or deaerated).

$$1/2H_2O_2 + h\nu \xrightarrow{k_f} HO^\bullet$$

$$HO^\bullet + Ade \xrightarrow{k_{bim}} Ade-8-OH^\bullet + \text{other adducts}$$

$$(k_{bim} = 4.3\times10^9\ M^{-1}\ s^{-1})$$

$$Ade-8-OH^\bullet \xrightarrow{k_1} 8-OH-Ade$$

(without added oxidants)

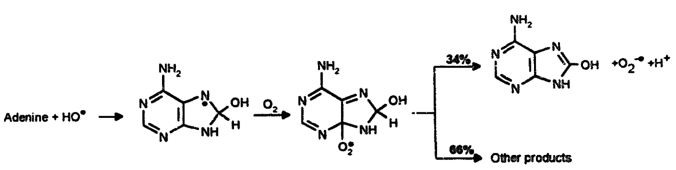
$$Ade-8-OH^\bullet \xrightarrow{k_2} \text{other products}$$

$$Ade-8-OH^\bullet + Fe^{III}(CN)_6^{3-} \xrightarrow{k_3} 8-OH-Ade$$

$$+Fe^{II}(CN)_6^{4-} + H^+ \quad (k_3 = 6\times10^9\ M^{-1}\ s^{-1})$$

$$[8-OH-Ade] = tk_0\frac{k_1 + k_3[Fe^{III}(CN)_6^{3-}]}{k_1 + k_2 + k_3[Fe^{III}(CN)_6^{3-}]} \qquad (1)$$

k_0 is defined as the rate at which the $HO^\bullet$ radicals produced add to the C(8) position of adenine, in concentration/time, and is numerically equal to k_f multiplied by 0.18, which is the fraction of $HO^\bullet$ radicals which add to C(8) in adenine [6]. In separate experiments, it was found that H_2O_2 photol-



Scheme 1.

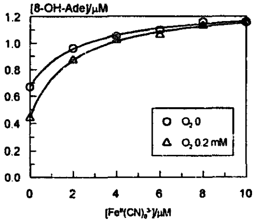


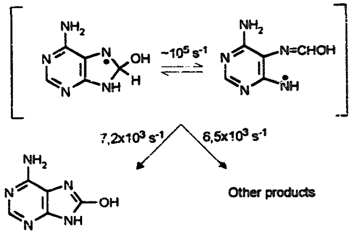
Fig. 3. Effect of the addition of ferricyanide on the formation of 8-OH–Ade after 10 min of UV photolysis of an aqueous solution containing 0.5 mM adenine and 1.0 mM H_2O_2 at pH 7 (aerated or deaerated). The superimposed curves represent the numerical fitting to the experimental data according to Eq. (1).

ysis, under these conditions, is very slow. Only at closer distances to the lamp (2.5 cm) was a 5% decrease in H_2O_2 concentration observed after 20 min. Thus k_f is approximately constant during photolysis.

Expression (1) was numerically fitted to the experimental data and is shown as a curve in Fig. 3 for deaerated solution. The plateau is numerically equivalent to tk_0, and was found to be 1.28 ± 0.02 μM for deaerated solution. By taking the ratio between the yields of 8-OH–Ade formed via oxidation by oxygen only (aerated solution, $[\mathrm{Fe^{III}(CN)_6^{3-}}]=0$) and by ferricyanide (plateau, for deaerated solution), the value found (34%) is a good estimate of the ratio of peroxyl radicals which eliminate superoxide anion with the consequent formation of 8-OH–Ade (Scheme 1).

The values found by numerical fitting for k_1 and k_2 are $7.2\times10^3\ \mathrm{s^{-1}}$ and $6.5\times10^3\ \mathrm{s^{-1}}$ respectively. Their sum can be regarded as a global pseudo-first-order constant for the decay of Ade–8-OH• into either 8-OH–Ade or other products. This value ($1.3\times10^4\ \mathrm{s^{-1}}$) is clearly smaller than that of a well-known unimolecular reaction undergone by Ade–8-OH•, i.e. imidazole ring opening, which occurs with a rate constant of $1.3\times10^5\ \mathrm{s^{-1}}$ [13]. The results show that the increase in the yield of 8-OH–Ade caused by ferricyanide is about ten times higher than expected from its rate constant for the reaction with Ade–8-OH•, compared with the rate constant for unimolecular ring opening. This result cannot be explained unless it is assumed that the imidazole ring opening is a reversible reaction (Scheme 2), as has been suggested previously [6].

Another unexpected result is the observation of a similar effect on addition of ferricyanide to aerated solutions, i.e. an increase in the yield of 8-OH–Ade (Fig. 3, for aerated solution). Under such conditions, Ade–8-OH• radicals are captured much more rapidly by oxygen ($[O_2]=0.2$ mM) than by $\mathrm{Fe^{III}(CN)_6^{3-}}$ (concentration in the range 0–10 μM); therefore the increase in the yield of 8-OH–Ade must be explained by the fact that ferricyanide also oxidizes the peroxyl radical mentioned above by the following reaction



Scheme 2.

$$\mathrm{Ade-8-OH-O_2^{\bullet}+Fe^{III}(CN)_6^{3-}\rightarrow 8-OH-Ade+Fe^{II}(CN)_6^{4-}+O_2+H^+} \quad (2)$$

It should be mentioned that the reduction potential for the couple ferricyanide/ferrocyanide is +0.336 V [14] and that of $O_2/HO_2^{\bullet}$ is −0.05 V [15]. It is also noteworthy that, in this case, the value found for the limiting plateau, 1.28 ± 0.02 μM, which corresponds to the rate of Ade–8-OH• formation, is similar to that obtained in deaerated solution, indicating that, for $[\mathrm{Fe^{III}(CN)_6^{3-}}]\geq 10$ μM, all the initially formed Ade–8-OH• adducts are quantitatively oxidized to 8-OH–Ade whether or not they are captured by oxygen.

4. Conclusions

The formation of 8-OH–Ade, from the reaction of HO• generated by the UV photolysis of hydrogen peroxide with adenine, is partially inhibited by oxygen, in spite of the reducing properties of the adduct precursor to this product, Ade–8-OH•. This is explained by the fact that the peroxyl radical, resulting from the addition of oxygen to Ade–8-OH• (Ade–8-OH–$O_2^{\bullet}$), eliminates superoxide anion at a rate which accounts, at most, for 34% of the sum of the rates of all the processes by which Ade–8-OH• decays. Ferricyanide anion promotes the oxidation of Ade–8-OH• more efficiently than oxygen since it reacts by electron transfer. Furthermore, this inorganic oxidant can oxidize Ade-8-OH-$O_2^{\bullet}$. Since oxygen is the most important oxidant under biological conditions, these results suggest that, after $HO^{\bullet}$ attack at the C(8) position of adenine, most of the damage is likely to lead to the degradation of the purine skeleton, instead of the formation of the 8-hydroxy derivative.

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