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Fast repair of deoxythymidine radical anions by two polyphenols: rutin and quercetin

Chenyang Zhao[a], Yimin Shi[a], Wenfeng Wang[b], Zhongjian Jia[c], Side Yao[b], Botao Fan[d], Rongliang Zheng[a,*]

[a]*School of life Sciences, Lanzhou University, Lanzhou 730000, China*
[b]*Laboratory of Radiation Chemistry, Shanghai Institute of Nuclear Research, Chinese Academy of Sciences, Shanghai 201800, China*
[c]*State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, China*
[d]*Institute de Topologie et de Dynamique des Systems, University Paris 7, Paris 75005, France*

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Abstract

The effects of rutin and quercetin on the repair of the deoxythemindine radical anion ($dT^{\bullet -}$) were studied using the technique of pulse radiolysis. The radical anion of dT was formed by the reaction of hydrated electron with dT. After pulse irradiation of nitrogen-saturated aqueous solutions containing dT, 0.2 M *t*-BuOH and either rutin or quercetin, the initially formed $dT^{\bullet -}$, detected spectrophotometrically, rapidly decayed with the concurrent formation of the radical anion of rutin or quercetin. The results indicated that $dT^{\bullet -}$ can be rapidly repaired by rutin or quercetin. The rate constants of the repair reactions were determined to be 3.1 and 4.1×10^9 M^{-1} s^{-1} for rutin and quercetin, respectively. With substitution by glycosyl groups at C_3–OH being neighbor to C_4 keto group, which is the active site for electron transfer, rutin has a lower repair reaction rate constant toward $dT^{\bullet -}$ than quercetin. Together with findings from our previous studies, the present results demonstrated that nonenzymatic fast repair may be a universal form of repair involving phenolic antioxidants.

Keywords: DNA damage; Fast repair; dT radical anion; Polyphenol; Electron transfer; Pulse radiolysis

1. Introduction

DNA damage is generally regarded as carcinogenic and actively participates in the process of tumor initiation, promotion and progression. DNA damage also can be induced by ionizing radiation, UV light and a variety of chemical agents as well as reactive oxygen species produced in the normal metabolism as byproducts. Ionization can induce DNA damage through two ways: directly occurs within the DNA itself and generates base radical cations and base radical anions [1] or indirectly produces hydrated electrons (e_{aq}^-) and hydroxyl radicals ($OH^{\bullet}$) through ionization of water in close vicinity to DNA which easily react with DNA and induce DNA damage. DNA bases have a very high intrinsic reactivity with e_{aq}^- [2] and pyrimidine is a much better electron acceptor than purine. The results of electron paramagnetic resonance studies demonstrated that base radicals including radical cations and anions, being primary and major products of ionizing radiation as well as e_{aq}^- addition, can induce strand breaks [3,4] or form stable base lesions [2]. The enzymatic repair systems in both prokaryotic and eukaryotic cells can efficiently repair DNA damage including DNA strand breaks and stable base lesions [5], but they are not perfect. It is true that some DNA damage always escape from enzymatic repair systems, even existing SOS process allowing misrepaired products present [6]. Moreover, during the process of aging or disease, metabolism of xenobiotics, DNA damage may be exacerbated, most of the repair capacity decreases, resulting in the accumulation of DNA damage and increase in mutation frequency. This means that DNA damage always exists and probably leads to degenerative disease

* Corresponding author. Tel.: +86-931-8912563; fax: +86-931-8912561.
E-mail address: zhengrl@lzu.edu.cn (R. Zheng).

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including cancer and aging. So it is necessary to look for an approach to supplement the inadequate repair capacity of cells by scavenging reactive oxygen species or hydrated electrons prior to damaging DNA or repairing damaged DNA before DNA replication.

Based on this understanding, much attention has been focused on the scavenging activity of antioxidants to reduce the risk of cancers and other chronic diseases. The e_{aq}^- scavenging potential of some compounds has been investigated. Cai *et al*. [7] reported that some flavonoids and phenolic acids can react with e_{aq}^- at close diffusion control rate (10^9 to 10^{10} M^{-1} s^{-1}) implicating that these compounds may act as efficient scavengers of e_{aq}^-. Our study also showed that phenylpropanoid glycosides and their analogs are potent e_{aq}^- scavengers (no published data). With direct effect of radiation, however, because ionization occurs within the DNA itself, DNA damage caused thereby cannot be prevented by antioxidants or e_{aq}^- scavengers. In indirect effect, because of very high reactivity of the primary products of ionization of water, e_{aq}^- and $OH^\bullet$ [2] and the much higher concentration of biomolecules than scavengers in cells, reactions of e_{aq}^- or $OH^\bullet$ with biomolecules are very difficult to prevent *in vivo* unless the concentration of scavengers is sufficiently high. Therefore, a more effective and feasible strategy to prevent mutation should be elimination or neutralization of DNA radicals either resulting from ionization of DNA or generated secondarily by the attack of $OH^\bullet$ and e_{aq}^-, that is nonenzymatic repair of DNA damage or fast repair [8].

With this consideration, the possible influence of some chemicals in nonenzymatic repair of DNA damage have been explored. O'Neill reported the fast repair effects of endogenous antioxidants, such as thiols and ascorbate towards oxidizing hydroxyl radical adducts of dGMP and dG with high rate constants (3.6×10^7 to 8.4×10^8 M^{-1} s^{-1}) [9,10]. Jiang *et al*. [11] showed that hydroxycinnamic acid derivatives can fast repair hydroxyl radical adduct of dGMP. The rapid electron transfer reaction between thymidine anion and caffeic acid was reported by Zou *et al*. [12]. We found fast repair activities of polyphenols, phenylpropanoid glycosides and their analogs, towards hydroxyl radical adducts of dGMP, dAMP [13–16], poly A, poly G, ssDNA and dsDNA (doctoral thesis of Shi YM), thymine radical anion [17,18], TMP radical anion [19], radical cations of dAMP, dGMP and dCMP [20]. The mechanism of fast repair was elucidated as either reduction or oxidation of DNA radicals. However, the evidences are still insufficient to establish the universality of fast repair of DNA damage by chemicals.

The pharmacological significance of polyphenols was recognized very early. These polyphenols are known to scavenge free radicals [21], have beneficial action in cardiovascular disorders [22], inhibit H_2O_2-induced V79 cell death, prevent DNA single strand breakage [23] and repair the radical cations of dCMP and poly C [24]. However, their potential to rapidly repair DNA radical anion has not been investigated.

The current study focuses on the repair of dT radical anion ($dT^{\bullet -}$) by employing two representative flavonoids: rutin (R) and quercetin (Q).

2. Materials and methods

2.1. Materials

Deoxythymidine (dT), rutin and quercetin were purchased from Sigma. All other chemicals were purchased from Shanghai Biochemical Corporation.

All solutions were prepared with triple distilled water, saturated with high purity nitrogen by bubbling for 20 min before irradiation and buffered with phosphate (2 M, pH 7.0). All experiments were carried out at room temperature.

2.2. Pulse radiolysis

Pulse radiolysis experiments were conducted using a linear accelerator providing 8 MeV electron pulse of 8 ns duration. The thiocyanate dosimeter was used for dose determination, assuming $\varepsilon_{(SCN)^-} = 7600$ dm^3 mol^{-1} cm^{-1} at 480 nm in nitrous oxide saturated 10 mM KSCN aqueous solution. The detailed descriptions of the pulse radiolysis equipment and experimental conditions were given elsewhere [25]. In these experiments, the average dose per pulse is 14 Gy.

2.3. Generation of hydrated electron

In aqueous solutions upon pulse radiolysis, hydrated electrons (e_{aq}^-), $OH^\bullet$ and hydrogen atoms ($H^\bullet$) are produced with G's (μmol J^{-1}) of 0.29, 0.29 and 0.06, respectively [26]. $OH^\bullet$ is scavenged by *t*-BuOH:

$$H_2O \rightarrow OH^\bullet + e_{aq}^- + H^\bullet \quad (1)$$

$$t\text{-BuOH} + OH^\bullet \rightarrow t\text{-BuOH}(-H)^\bullet + H_2O \quad (2)$$

3. Results

3.1. Transient optical absorption spectrum of dT radical anion

On pulse irradiation of 2 mM dT aqueous solution saturated with nitrogen at pH 7.0, a transient optical absorption spectrum arising from reaction of e_{aq}^- with dT was observed, and was characterized by an optical absorption maximum at 340 nm (Fig. 1). This transient absorption spectrum was assigned to dT radical anion ($dT^{\bullet -}$) [17]. The transient optical absorption reached a maximum at 1 μs after the pulse irradiation (Fig. 1, inset).

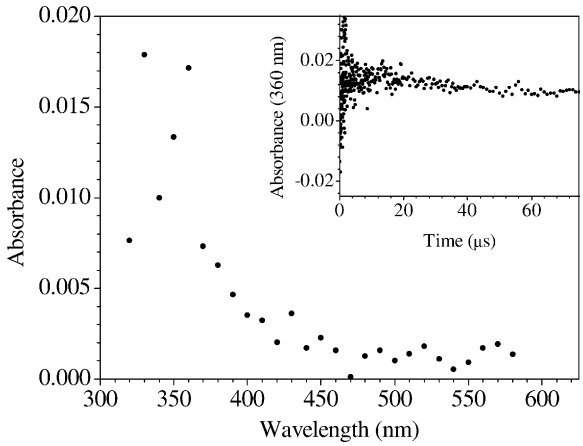


Fig. 1. Transient absorption spectrum upon pulse radiolysis of 2 mM dT (at 4 μs) aqueous solution containing 200 mM *t*-BuOH and saturated with N_2 at pH 7.0. Inset: the buildup trace of absorption at 340 nm.

3.2. Transient absorption spectra of radical anions of the tested polyphenols

In the pulse radiolysis of 0.1 mM rutin aqueous solution containing 200 mM *t*-BuOH and saturated with nitrogen, a transient absorption spectrum appeared and was characterized by a maximum absorption at 380 nm (Fig. 2A). The optical absorption reached a maximum after 5 μs (Fig. 2A, inset).

The transient absorption spectrum of product of reaction between hydrated electron and quercetin was also observed with similar condition (Fig. 2B).

With regard to the reactions of polyphenols with e_{aq}^-, the benzoyl or the styryl keto group of polyphenols is the site on which e_{aq}^- attacked and resulting in ketyl radical ions, which are radical anions of polyphenols [7,27]. Therefore, the transient absorption spectra shown in Fig. 2 should be assigned to radical anion of rutin and quercetin ($R^{\bullet -}$ and $Q^{\bullet -}$), respectively.

3.3. Repair reaction of dT radical anion by tested polyphenols

At 1 μs after pulse radiolysis of 2 mM dT aqueous solution containing 0.1 mM rutin, 200 mM *t*-BuOH and saturated with nitrogen at pH 7.0, a transient absorption spectrum was observed (Fig. 3A(a)). This spectrum is same as that of $dT^{\bullet -}$, therefore the spectrum is assigned to $dT^{\bullet -}$. At 15 μs after the pulse irradiation, a new optical absorption with $\lambda_{max} = 380$ nm grows in concurrence with the disappearance of that of $dT^{\bullet -}$ (Fig. 3A(b)). Based on its similarity with rutin radical anion ($R^{\bullet -}$), the new transient absorption spectrum is assigned to $R^{\bullet -}$. This change of transient absorption spectrum is due to one electron transfer from $dT^{\bullet -}$ to R by which $dT^{\bullet -}$ is restored to dT and then $R^{\bullet -}$ is formed.

The analogous results were observed on pulse radiolysis of 2 mM dT aqueous solution containing 0.1 mM quercetin, 200 mM *t*-BuOH and saturated with nitrogen at pH 7.0 (Fig. 3B).

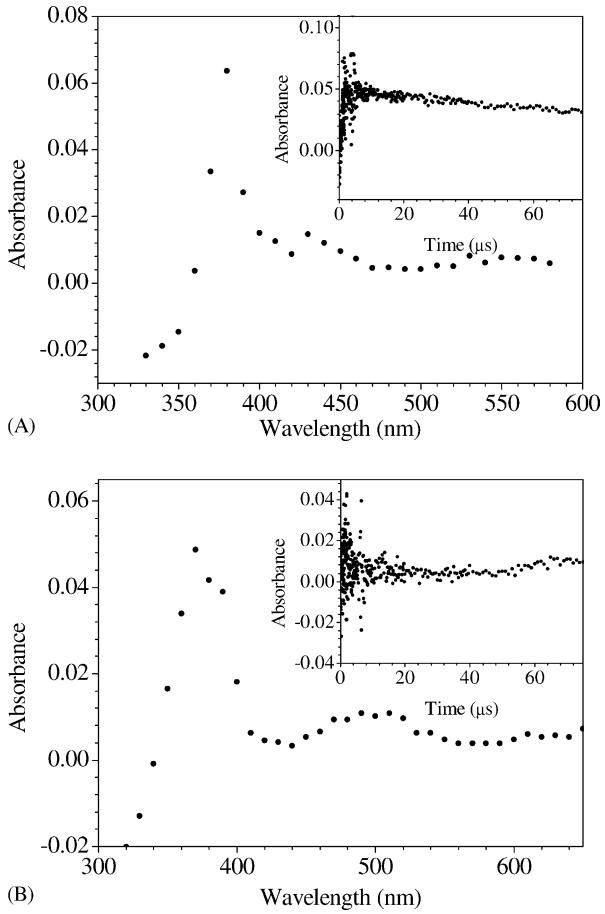


Fig. 2. Transient absorption spectra upon pulse radiolysis of 0.1 mM polyphenols aqueous solution containing 200 mM *t*-BuOH and saturated with N_2 at pH 7.0. (A) Rutin, at 4 μs; (B) quercetin, at 2 μs. Inset: the buildup trace of absorption at (A) 380 nm and (B) 370 nm.

Fig. 3A inset showed the growth of $R^{\bullet -}$ on pulse radiolysis of 2 mM dT aqueous solution containing 0.1 mM rutin, 200 mM *t*-BuOH and saturated with nitrogen. The curve represents the change of absorption of $R^{\bullet -}$ at 440 nm with time after the pulse radiation. The growth of absorbance follows first order kinetics, from which the apparent rate constant for the formation of $R^{\bullet -}$ by the repair reaction, k_{app} was determined. From the linear dependence of k_{app} on the concentration of rutin (0.02–0.1 mM), the rate constant (k) for electron transfer from $dT^{\bullet -}$ to R was determined. The rate constants of reactions of tested polyphenols with $dT^{\bullet -}$ were deduced and shown in Table 1.

Table 1
The rate constants of repair reaction of dT radical anion by polyphenols

	$k/10^8$ M^{-1} s^{-1}
Rutin	3.1
Quercetin	4.1

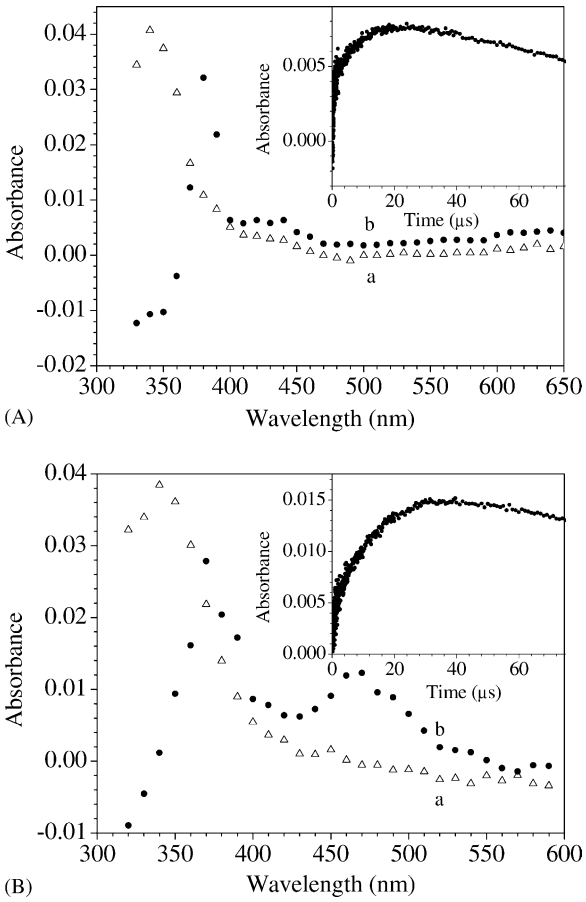


Fig. 3. Transient absorption spectra upon pulse radiolysis of saturated with N_2 2 mM dT aqueous solution containing 200 mM *t*-BuOH and (A) 0.1 mM rutin, a: 1 μs, b: 15 μs; (B) 0.1 mM quercetin, a: 1 μs, b: 35 μs. Inset: the buildup trace of absorption at (A) 440 nm and (B) 460 nm.

4. Discussion

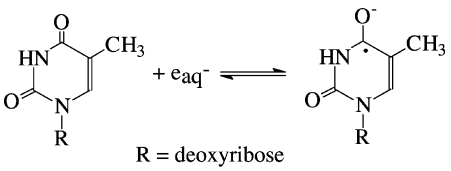
In principle, there are two parallel reactions competing in the above repair system:

$$dT + e_{aq}^- \rightarrow dT^{\bullet -} \ldots k_1 \quad (3)$$

$$R\,(\text{or}\,Q) + e_{aq}^- \rightarrow R\,(\text{or}\,Q)^{\bullet -} \ldots k_2 \quad (4)$$

However, according to the mechanism of competition reaction, the reaction probability (P) of dT with e_{aq}^- is calculated by $P = k_1[dT]/(k_1[dT] + k_2[R])$ to be 0.97 (97%). Therefore, almost all e_{aq}^- predominantly reacts with dT to form dT radical anion.

Concerning the reaction of hydrated electron with dT, the addition of hydrated electron to C_4 produces dT radical anion (Eq. (5)). The product is a reducing radical.

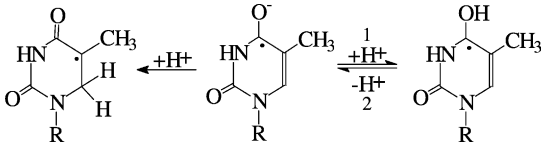


(5)

Although rutin and quercetin have long been known as efficient natural antioxidants, with benzoyl or styryl keto, they may act as electron acceptors, can also react with dT radical anion (Eq. (6)).

$$dT^{\bullet -} + R\,(Q) \rightarrow dT + R\,(Q)^{\bullet -} \quad (6)$$

However, it is generally suggested that in aqueous solution dT radical anion can undergo reversible protonation at O_4 whose rate constant was $k_{+H^+} = 2.6 \times 10^{10}\ M^{-1}\ s^{-1}$ and $k_{-H^+} = 1 \times 10^{10}\ M^{-1}\ s^{-1}$, and can undergo irreversible protonation at C_6 at rate constants of $5 \times 10^3\ M^{-1}\ s^{-1}$ (Eq. (7)) [28].



(7)

Obviously, the rate constants of reversible protonation are higher than that of repair reaction of $dT^{\bullet -}$ by rutin and quercetin. Thus, in the above repair system the protonation reaction would compete with repair reaction. Fortunately, the protonation reaction of $dT^{\bullet -}$ is reversible, and on the other hand, $dT^{\bullet -}$ is a weak base and only partly was protonated in neutral aqueous solution. Therefore, the reaction of dT radical anion with rutin and quercetin can destroy the reversibility, and leads to the equilibrium (Eq. (6)) toward dT radical anion, and finally reaction 2 is advantageous in competition between reactions 1 and 2. Therefore, it is necessary to suppress the protonation at C_6, which would result in generation of stable dihydrothymine. It is proposed that rutin and quercetin are able to inhibit protonation that can cause further damage to cell, implicating a potential repair activity.

For polyphenols acting as electron acceptors, their benzoyl or styryl keto group is the active site, whichever bulky group being neighbor to the keto would have negative effects on their electron reception capacities. With glycosylation at C_3–OH, rutin has a lower electron reception capacity than quercetin [7]. The current study also shows that the rate constant of fast repair reaction of dT radical anion by rutin is lower than that by quercetin (Table 1).

Concerning cellular DNA, a two-component hypothesis has been developed. According to this hypothesis, the electron loss centers (radical cations) end up with the purines, particularly with the guanine moiety, whereas the final site of deposition of the ejected electron is with the pyrimidines, particularly with thymine [2]. In other words, the two-component hypothesis implies that in DNA there are mechanisms of electron and positive hole transfer by which the initially generated and randomly distributed electron gain and loss centers are tunneled into the T and G "traps," respectively. Therefore, it is reasonable to say that by repairing dT radical anion, rutin and quercetin can repair indirectly other base radical anions produced in cellular DNA by radiation, protecting DNA from strand breaks.

As the product of protonation, dihydrothimine present in DNA is generally considered to be relatively innocuous in terms of constituting a replicative block. However, it appears

that accumulation of a large number of dihydrothymines in DNA may still be a disadvantage for cells since they would generate a significantly detectable level of termination of polymerization *in vivo*. Furthermore, the *N*-glycosylic bond of dihydrothymine is more susceptible to hydrolysis than thymine, therefore apurine/aprymidine (AP) sites would be formed more frequently from dihydrothymine than from thymine. It is more important for repair enzymes to recognize and remove dihydrothymine [29].

Cai *et al*. [7] reported that some flavonoids and phenolic acids including rutin and quercetin can react with hydrated electron at close to diffusion control rate meaning they are efficient scavengers of hydrated electrons. So, rutin and quercetin not only fast repair DNA damage caused by radiation, but also scavenge hydrated electron, thereby, protecting DNA from hydrated electron attack. Therefore, rutin and quercetin may act as efficient radioprotectors and therapeutical agents for the diseases related with DNA damage.

The result of the current study together with that of our previous studies demonstrate that nonenzymatic fast repair may be a universal form of repair involving phenolic compounds.

Acknowledgments

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