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Repair effect of phenylpropanoid glycosides on thymine radical anion induced by pulse radiolysis

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Abstract

Repair effects on thymine radical anion by six phenylpropanoid glycosides (PPGs), isolated from *Pedicularis* species, were studied using pulse radiolysis method. The thymine radical anion was produced by the reaction of hydrated electron with thymine. PPGs were added into the thymine solution saturated with N_2. Kinetic analysis showed that transient absorption spectrum of thymine radical anion formed at first, and then after several microseconds of pulse radiolysis changed to that of PPG radical anion. The evidence indicated that thymine radical anion was repaired through one-electron transfer between the radical anion and PPG. Electrophilic phenyl-substituted unsaturated carboxylic group containing in PPGs' structure was able to capture electron from thymine radical anion before it undergo reversible protonation. The reaction rate constants of electron transfer from thymine radical anion to PPGs were within $1.16–2.29 \times 10^9$ dm^3 mol^{-1} s^{-1}. © 1997 Elsevier Science B.V.

Keywords: DNA base repair; Phenylpropanoid glycosides; Thymine radical anion; Electron transfer; Pulse radiolysis

1. Introduction

DNA base damage may be involved in many pathologic processes ([1–3]), therefore, there is an increasing interest in the potential role of DNA base repair in anti-mutagenesis, anti-carcanogenesis, anti-aging and protection of reproductive cell death, etc. During water radiolysis, the main reactive radicals are hydrated electron (e_{aq}) and hydroxyl radical ($OH^{\cdot}$). Hydroxyl radical has been implicated as the DNA-damaging species by a number of investigations ([4–8]). A lot of experimental studies implicate that e_{aq} produced in mammalian cell is able to attack protein and DNA ([9]). The reaction of e_{aq} with ribonuclease causes fragmentation of peptide chain to lead to inactivation of the enzyme ([10]). Hydrated electron reacts exclusively with bases in DNA to form base radical anion in aqueous solution ([9]). Considering the scale of electron affinity of the different nucleotides, the pyrimidine are found to be much better electron acceptors than the purine ([11]). Pyrimidine radical anion easily protonate to produce stable base lesion ([9]). These damages are considered the critical lesion giving rise to chromosomal

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aberrations that finally result in mutation, aging and cancer ([12,13]). Fortunately, DNA repair enzymes have been involved in the repair process of DNA damage ([14]). The repair enzymes continually scan DNA sequence mistake, slice out damaged pieces and patch up gaps to preserve genetic information. Meanwhile, some endogenous antioxidants prove to protect DNA against radiation through a favorable influence on enzymatic repair ([15]). Cerutti and co-workers demonstrate that antioxidant defenses appears to be important for the cellular resistance to oxidant-induced damage to the genome and cell killing ([16]). They believed that the balance between several antioxidant enzymes, rather than the activity of a single component, determines the degree of protection ([17,18]). However, there is insufficient evidence to prove that natural antioxidants occurring in plants can play a protection role in DNA damage through reaction with damaged bases.

Phenylpropanoid glycosides (PPGs), polyphenolic compounds, were isolated from *Pedicularis* species. As a folk medicinal herb, *Pedicularis* is used in folk medicine as cardiac-tonic of collapse, exhaustion, spontaneous sweating, seminal emission and senility, invigorate circulation of blood, aid digestion, full of vitality, relieve uneasiness of body and mind ([19]). PPGs have been reported to have antiviral ([20]), antiplatelet activities ([21]), and to inhibit leukotriene B_4 formation ([22]). Our studies showed that PPGs were able to scavenge reactive oxygen radicals ([23,24]), to inhibit lipid peroxidation ([25,26]), to chelate ferrous ions (unpublished results) and to inhibit the growth of tumor cells ([27,28]). In the present work, the protective effect of PPGs on thymine radical anion were studied using the pulse radiolysis in aqueous solution.

2. Materials and methods

2.1. Materials

PPGs used in the study are verbascoside (VER), leucosceptoside A (LEU), martynoside (MAR), pedicularioside A (PED-A), pedicularioside M (PED-M) and pedicularioside N (PED-N), and have high purity (98%). They were extracted from *Pedicularis spicata* and *plicata* ([29,30]). Their structures were shown in Fig. 1. Thymine was purchased from Sigma. Methyl propan-2-o (*t*-BuOH) was redistilled. All solutions were made in triply distilled water, and saturated with high purity N_2. All experiments were carried out at room temperature.

2.2. Pulse radiolysis

Pulse radiolysis experiment was conducted by using a linear accelerator providing 10 MeV electron pulse with a duration 8 ns. The pulse was determined by thiocyanate dosimeter containing 10 mM KSCN solution saturated with nitrous oxide dosimetery of electron by taking $\epsilon_{(SCN)_2^-} = 7600\ dm^3\ mol^{-1}\ cm^{-1}$ at 480 nm. In this experiment, the maximum dose of a single pulse is 20 Gy. The detail description of set up of pulse radiolysis equipment and experimental condition were described in Ref. [31].

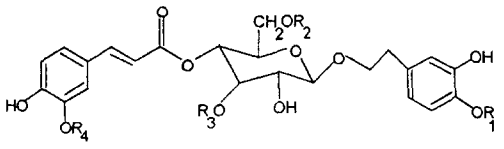


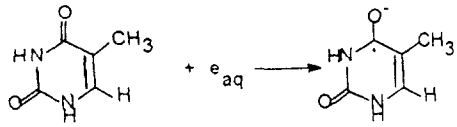
Fig. 1. The structures of phenylpropanoid glycosides.

verbascoside	$R_1=R_2=R_4=H$		R_3 = rhamnosyl	
leucosceptoside A	$R_1=R_2=H$	$R_4=CH_3$	R_3 = rhamnosyl	
martynoside	$R_2=H$	$R_1=R_4=CH_3$	R_3 = rhamnosyl	
pedicularioside A	$R_1=R_4=H$		R_3 = rhamnosyl	R_2 = apiosyl
pedicularioside M	$R_1=H$	$R_4=CH_3$	R_3 = rhamnosyl	R_2 = apiosyl
pedicularioside N	$R_1=R_4=CH_3$		R_3 = rhamnosyl	R_2 = apiosyl

3. Results

3.1. Transient absorption spectra of thymine and PPGs radical anions

Aqueous solution of 1 mM thymine saturated with N_2 containing 20 mM *t*-BuOH as scavenger of hydroxyl radical was irradiated by pulse electron beam. A transient absorption spectrum was observed and was characterized by maximum absorption at 340 nm (Fig. 2). Since output of hydrogen atom produced at first by pulse radiolysis is very low, the primary radical in the solution is mainly e_{aq}^-, which attacks the carbonyl functional group of thymine to form thymine radical anion ($T^{\cdot -}$) [Eq. (1)] ([32]). So the transient absorption shown in Fig. 2 should be assigned to that of $T^{\cdot -}$. The result was in agreement with that of Hayen ([32]). The rate constant of the reaction is 1.7×10^9 dm^3 mol^{-1} s^{-1} ([33]).



(1)

After pulse radiolysis of N_2-saturated aqueous solution containing 0.1 mM PPG and 20 mM *t*-BuOH, transient absorption spectra appeared and are characterized by maximum absorption at 370 nm or 380 nm (Fig. 3). Electrophilic phenyl-substituted unsaturated carboxylic group of PPGs can be attacked by

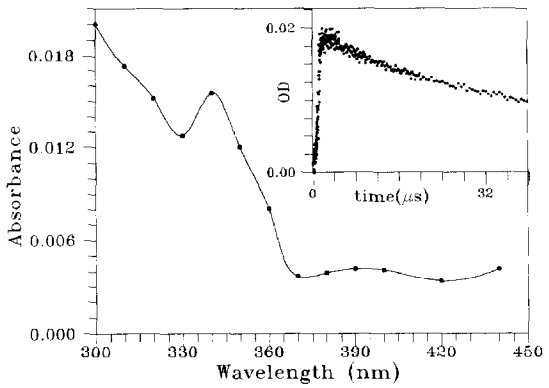


Fig. 2. The transient absorption spectrum at 2 μs after pulse radiolysis of 1 mM thymine and 20 mM *t*-BuOH aqueous solution saturated with N_2. Inset: growth trace of absorption at 340 nm.

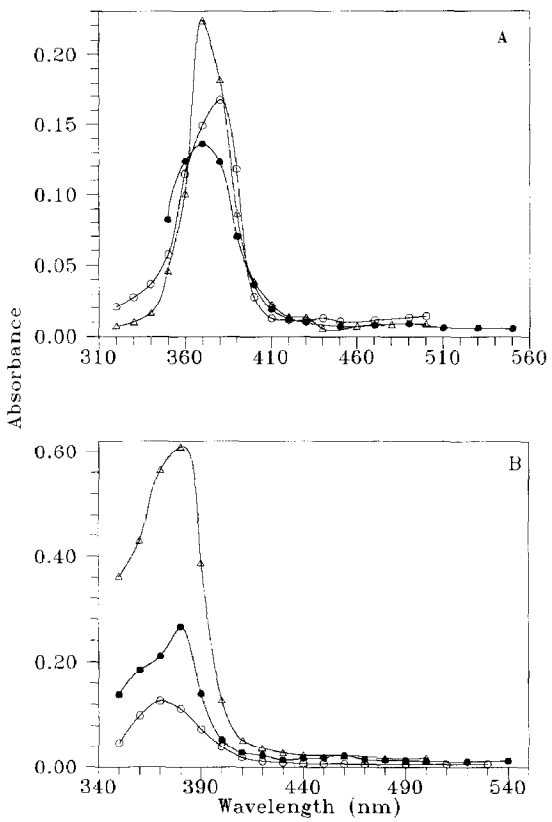


Fig. 3. The transient absorption spectra at 5 μs after pulse radiolysis of 0.1 mM PPG and 20 mM *t*-BuOH aqueous solution saturated with N_2. (A) – · – verbascoside (λ_{max} = 370 nm); – ○ – leucosceptoside A (λ_{max} = 380 nm); – △ – martynoside (λ_{max} = 370 nm). (B) – · – pedicularioside A (λ_{max} = 380 nm); – ○ – pedicularioside M (λ_{max} = 370 nm); – △ – pedicularioside N (λ_{max} = 380 nm).

e_{aq}^- to form their radical anions ($PPG^{\cdot -}$). Therefore, the transient species in Fig. 3 are that of six $PPG^{\cdot -}$, respectively, which were produced by one-electron reduction after pulse radiolysis.

3.2. Reaction of $T^{\cdot -}$ with PPGs

N_2 saturated aqueous solution containing 1 mM thymine, 0.06 mM VER and 20 mM *t*-BuOH was irradiated by pulse electron beam. A transient absorption spectrum appeared at 1 μs (Fig. 4A), which was the same as that of $T^{\cdot -}$ (Fig. 2). This indicated that e_{aq}^- generated in the above solution reacts firstly with thymine to form $T^{\cdot -}$. Forty microseconds later,

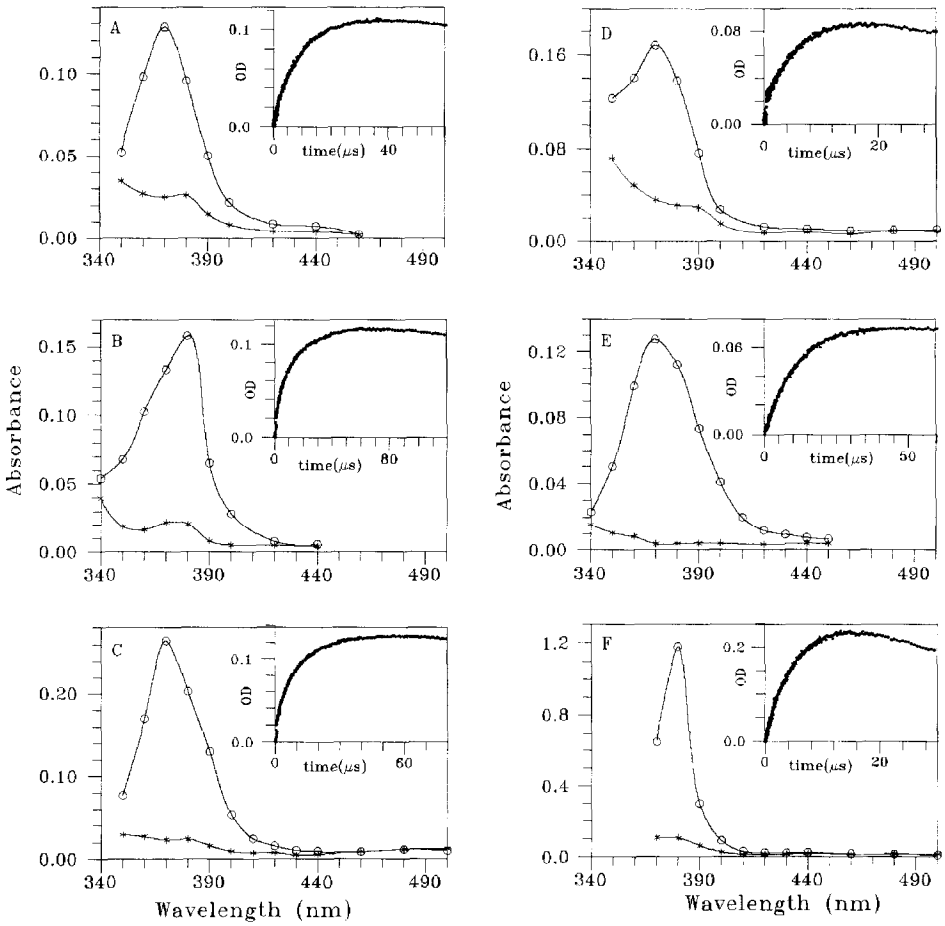


Fig. 4. Transient absorption spectra after pulse radiolysis of 1 mM thymine, 0.06 mM PPG and 20 mM *t*-BuOH aqueous solution saturated with N_2. (A) Verbascoside, (*) 1 μs, (○) 40 μs, inset: growth trace of absorption at 370 nm. (B) Leucosceptoside A, (*) 1 μs, (○) 45 μs, inset: growth trace of absorption at 380 nm. (C) Martynoside, (*) 1 μs, (○) 60 μs, inset: growth trace of absorption at 370 nm. (D) Pedicularioside A, (*) 1 μs, (○) 20 μs, inset: growth trace of absorption at 380 nm. (E) Pedicularioside M, (*) 1 μs, (○) 45 μs, inset: growth trace of absorption at 370 nm. (F) Pedicularioside N, (*) 1 μs, (o) 45 μs, inset: growth trace of absorption at 380 nm.

the transient absorption spectrum changed into a new one with maximum absorption at 370 nm. Based on its similarity with VER radical anion ($VER^{\cdot -}$) in Fig. 3A, the transient absorption spectrum should be assigned to that of $VER^{\cdot -}$. It is suggested that the change in absorption spectrum with different time was due to an electron transfer from $T^{\cdot -}$ to VER. After losing an electron, $T^{\cdot -}$ was restored to T. This process is different from the repair of enzymes in vivo.

$$T^{\cdot -} + VER \rightarrow VER^{\cdot -} + T \quad (2)$$

Arising from similar reaction, $T^{\cdot -}$ was repaired either by LEU, MAR, PED-A, PED-M or PED-N (Fig. 4B, C, D, E and F).

Fig. 4A inset represents the change of absorption of $VER^{\cdot -}$ at 370 nm with time after pulse radiolysis.

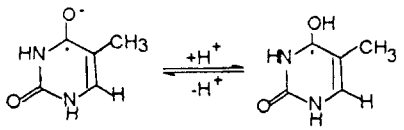
Table 1
The rate constants of electron transfer from thymine radical anion to PPG

PPGs	k ($dm^3\ mol^{-1}\ s^{-1}$)
Verbascoside	1.46
Leucosceptoside A	1.54
Martynoside	1.16
Pedicularioside A	2.29
Pedicularioside M	1.45
Pedicularioside N	1.33

By kinetic analysis of the growth trace of $VER^{\cdot -}$, it is found that the growth kinetics of $VER^{\cdot -}$ was first-order process. Variation of concentration of VER (0.01–0.1 mM), the rate constant of electron transfer was determined as 1.46×10^9 dm^3 mol^{-1} s^{-1}. The rate constants of electron transfer between $T^{\cdot -}$ and other PPGs were presented in Table 1.

4. Discussion

In general, it is suggested that $T^{\cdot -}$ produced in aqueous condition can undergo reversible protonation whose rate constant was $k_{-H^+} = 2.6 \times 10^{10}$ dm^3 mol^{-1} s^{-1} and $k_{+H^+} = 1 \times 10^{10}$ dm^3 mol^{-1} s^{-1} ([34]).



(3)

The rate constant of the protonation was higher than PPGs repair reactions. However, because the reaction of protonation was reversible, the reaction of PPGs with $T^{\cdot -}$ destroys the reversibility, and leads to the equilibrium (Eq. (3)) towards T, finally, reaction (2) has the advantage of competition between reactions (3) and (2). It is therefore suggested that PPGs are able to inhibit $T^{\cdot -}$ protonation which can cause further damaging in living system ([9]).

In the area of irradiated DNA damage, the question of which reaction probability of e_{aq} with thymine (T) or cytosine (C) is higher still remains unclear. However, because of the fact that T has the lowest unoccupied molecular orbital ([35]), one suggested that C may capture electron at first, and then electron transfer occurs in the direction C → T ([36]). Thus, the T moiety is the endpoint of electron. As the product of protonation, dihydrothymine present in DNA is relatively innocuous in terms of constituting a replicative block ([37]). It appears, however, that accumulation of a large number of dihydrothymines in DNA may still be a disadvantage for cells since in vivo they would generate a significantly detectable level of termination of polymerization. Further, the *N*-glycosylic bond of dihydrothymine is more susceptible to hydrolysis than thymine ([38]), therefore apurine/apyremidine (AP) sites would be generated more frequently from dihydrothymine than from thymine. It is more important that cells process repair enzymes that recognize and remove dihydrothymine, therefore dihydrothymine may have biological consequences ([37]). Since the present study demonstrated that PPGs are able to react with $T^{\cdot -}$ efficiently, PPGs should be able to suppress e_{aq} damage on DNA as well. Therefore, we suggested that PPGs may probably act as a series of efficient radioprotectors and therapeutical agents for the diseases related with DNA damage. Moreover, some studies showed the defective repair of base lesion was processed by DNA repair enzymes or other nuclear factors ([39,40]). Although the process of reaction PPG with $T^{\cdot -}$ is very different from the repair of enzymes, its protective activities may remedy the defect. Only a few studies have been reported on protective activity from herbal sources ([5,41]). The study offered the evidence.

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