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Fast repair of the poly G hydroxyl radical adduct by two phenylpropanoid glycosides

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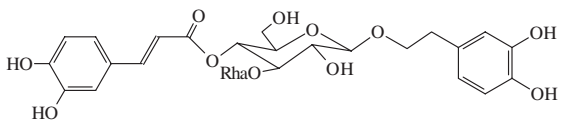
The repair activities and the reaction mechanisms of two phenylpropanoid glycosides (PPGs), isolated from a herb used in Chinese folk medicine, towards the oxidizing hydroxyl radical adduct of poly G were studied with a pulse radiolytic technique. On pulse irradiation of a nitrous oxide saturated 4 mM aqueous solution of poly G containing one of the tested compounds, the transient absorption spectrum of the hydroxyl radical adduct of poly G decays with the formation of that of the phenoxyl radical of the tested compound within several tens microseconds after the electron pulse irradiation. The results indicated that there was a repair reaction between the hydroxyl radical adduct of poly G and the compounds tested. The rate constants for the repair reactions of poly G hydroxyl radical adducts by verbasicoside and cistanoside C were determined to be 1.4 and $1.0 \times 10^9\ M^{-1} \cdot s^{-1}$, respectively.

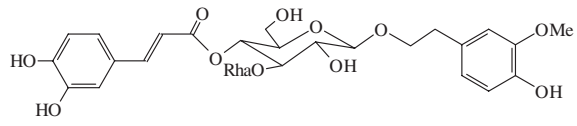
1. Introduction

Damage to DNA by oxygen radicals, if not repaired or misrepaired, can lead to either mutation or cellular dysfunction, and hence to a number of degenerative diseases including cancer and aging [1–3]. These radicals arise from metabolism of oxygen as well as from inflammatory events, ischemia, and xenobiotic metabolism [4–5]. Having extremely high activity, the hydroxyl radical can cause extensive damage to DNA including base modification, strand break and damage to the sugar moieties of nucleotides, as well as DNA-protein crosslinks and other types of damage [6]. Hydroxylated 2′-deoxyguanosines formed by $^{\bullet}OH$ addition to deoxyguanosine in DNA are the most abundant base lesion [7]. Hydroxylated 2′-deoxyguanosine, especially 8-hydroxyl-deoxyguanosine (8-OH-dG) which induces G → T transversion, is believed to be an important lesion of DNA leading to mutation. To avoid the cellular consequences of DNA damage, cells have evolved repair systems, which efficiently repair DNA damage including 8-OH-dG [8]. However, DNA damage induced by exogenous chemical agents and metabolic oxygen radicals occurs at a high rate exceeding the capacity of the cell for DNA repair [9], especially in the process of aging and in the case of some patients. This means that DNA damage always exists prior to DNA replication, and therefore that there is a possibility of mutation leading to degenerative diseases. So efforts are needed to look for ways in which either oxygen radicals are scavenged prior to their damaging DNA or damaged DNA is repaired to supplement the cell's inadequate repair capacity.

Much attention has been focused on the scavenging of ROS by antioxidants to prevent DNA from attack by them [10]. According to the principle of competition reaction, however, the effectiveness of scavenging of ROS by antioxidants may be limited in the cellular situation. This is because of the high reactivity of the hydroxyl radical and the much higher concentration of biomaterial than of scavengers in cells. Therefore, more feasible and efficient strategies for prevention of damage, e.g., mutations induced by hydroxyl radicals, should concentrate on the elimination or neutralization of the secondary bioradicals resulting from the attack of hydroxyl radicals, that is non-enzymatic repair of DNA damage or fast repair [11]. With regard to the non-enzymatic repair of DNA damage, the fast repair activities of thiols and ascorbate towards hydroxyl radical adducts of dGMP and dG were initially reported by O'Neill in 1983 [12]. The fast repair effects of hydroxycinnamic acid derivatives on hydroxyl radical adducts of dGMP were shown by Jiang et al. [13]. Our study showed that phenylpropanoid glycosides (PPGs) and their analogs can repair hydroxyl radical adducts of dGMP and dAMP [14–17], thymine radical anions [18–19], TMP radical anions [20] and deoxynucleotide radical cations [21]. However, so far no reports have been found indicating that the hydroxyl radical adducts of integral DNA rather than those of constituent parts of DNA can be repaired by either endogenous or exogenous antioxidants.



Verbasicoside



Cistanoside C (CIS C)

This is unfavorable for demonstrating the mechanism of non-enzymatic repair of DNA damage. The present study takes poly G as a model molecule to investigate the possibility of fast repair of hydroxyl radical adducts occuring in integral DNA.

Verbascoside (VER) and cistanoside C (CIS) have been isolated from *Pedicularis* species. *Pedicularis* is a herb of Chinese folk medicine used especially in Tibetan to treat malignant sores, collapse, exhaustion, spontaneous sweating, seminal emission and senility, and to invigorate circulation of blood, aid digestion, give vitality, and relieve uneasiness of body and mind. Some PPGs isolated from other plants have been reported to have antiviral [22], antitumor [23, 24], antioxidant [25, 26], antiplatelet and antibiotic [27], and immunosuppressive activities [28], and to inhibit formation of the 5-lipoxygenase products, 5-OH-6,8,11,14-eicosatetraenoic acid (5-HETE) and leukotriene B_4 [29]. Our studies showed that PPGs were able to inhibit the lipid peroxidation of mouse liver microsomes [30, 31], the antioxidation of linoleic acid in micelles [31], and the radical-induced hemolysis of erythrocytes [32], to chelate ferrous ions [23], to inhibit the growth of tumor cells [34], to scavenge hydroxyl radicals [35] and to enable tumor cell redifferentiation [36]. Their fast repair activities towards DNA damage have been verified in our laboratory with damage induced by oxidative stress [14–21].

In the present study, the repair effects of two phenylpropanoid glycosides on hydroxyl radical adducts of poly G were investigated by a pulse radiolytic technique.

2. Investigations and results

2.1. Transient absorption spectra of hydroxyl radical adducts of poly G

On pulse irradiation of 4 mM poly G solution saturated with nitrous oxide at pH 7.0, a transient absorption spectrum arising from reaction of $OH^{\bullet}$ with poly G was observed, and was characterized by an optical absorption maximum at 310 nm (Fig. 1). This transient absorption spectrum was assigned to the hydroxyl adduct of poly G (poly G-$OH^{\bullet}$). The transient optical absorption reached a maximum at 10 μs after the pulse irradiation. This result is in agreement with that of Scholes et al. [37] and Willson [38].

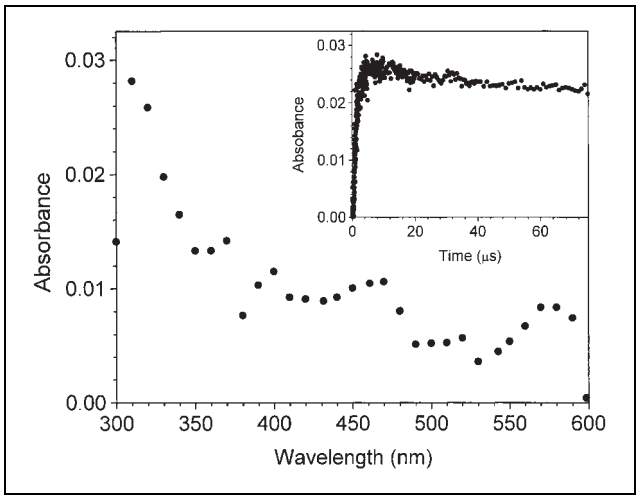


Fig. 1: Transient absorption spectra of hydroxyl radical adducts of poly G obtained by pulse radiolysis of its 4 mM nitrous oxide saturated neutral aqueous solution at 6 μs after pulse irradiation. Inset: the buildup trace of optical absorption, 320 nm

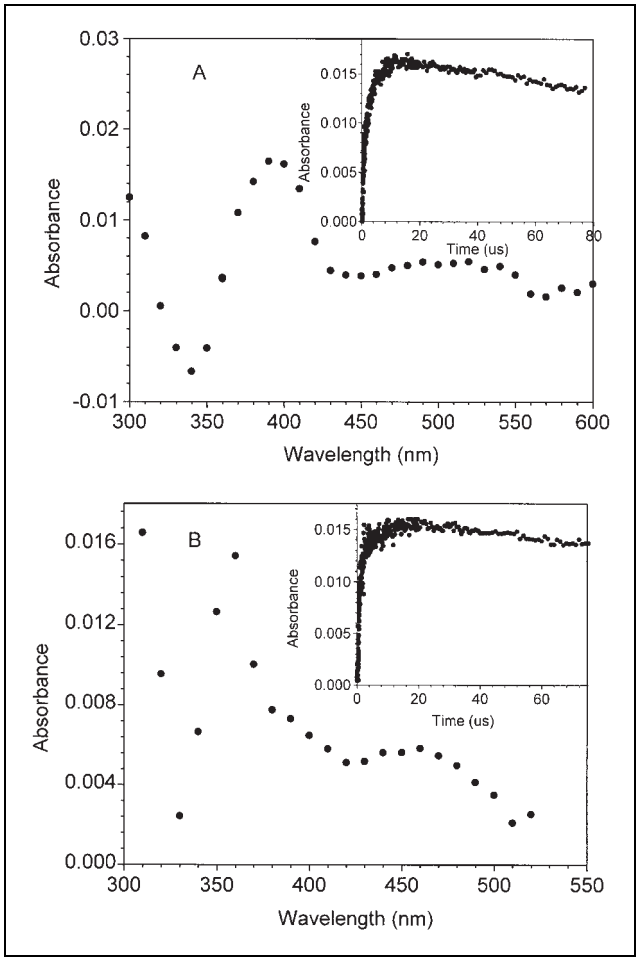


Fig. 2: Transient absorption spectra upon pulse radiolysis of 0.1 mM tested compounds aqueous solution saturated with N_2O at pH 7.0. A verbasicoside, at 10 μs; B cistanoside C, at 30 μs. Inset: the buildup trace of optical absorption, A 390 nm, B 360 nm

2.2. Transient absorption spectra of phenoxyl radicals of tested compounds

In the pulse radiolysis of 0.1 mM aqueous solution of VER saturated with nitrous oxide, a transient absorption spectrum appeared and was characterized by a maximum absorption at 390 nm (Fig. 2). The optical absorption reached a maximum after 10 μs. This transient absorption spectrum was assigned to the phenoxyl radical of VER (VER-$PhO^{\bullet}$) because of the fact that after hydroxyl radicals attack phenolic hydroxyl groups, phenoxyl radicals are formed [39].

The transient absorption spectrum of the phenoxyl radical of CIS C (CIS C−$PhO^{\bullet}$) with a maximum optical absorption at 360 nm was observed by the same process (Fig. 2).

2.3. Repair reactions of poly G-$OH^{\bullet}$ by tested compounds

On pulse radiolysis of 4 mM poly G aqueous solution containing 0.06 mM VER and saturated with N_2O, the transient absorption spectrum of poly G-$OH^{\bullet}$ was observed first (at 2 μs after pulse irradiation) (Fig. 3A-a). Thereafter, the transient absorption of VER-$PhO^{\bullet}$ grown and approached its maximum at 63 μs after the pulse irradiation concomitantly with the disappearance of that of poly G-$OH^{\bullet}$ (Fig. 3A-b). This result demonstrates that there is an electron transfer reaction between poly G-$OH^{\bullet}$

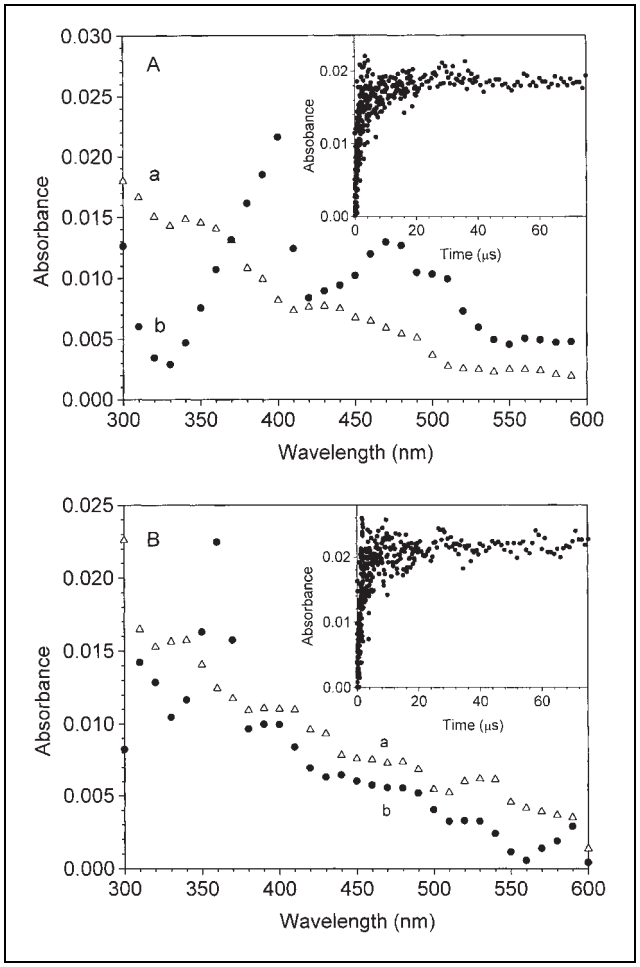


Fig. 3: Transient absorption spectra upon pulse radiolysis of 4 mM poly G aqueous solution saturated with N_2O and containing: A 0.06 mM verbasicoside, a 1 μs, b 63 μs; B 0.1 mM cistanoside C, a 5 μs, b 60 μs. Inset: the buildup trace of optical absorption, A 390 nm, B

and VER, therefore implicating VER in the repair effect on poly G-OH•.

The repair activities of CIS C towards poly G-OH• were also observed under the same conditions (Fig. 3B).

3. Discussion

With its extremely high reactivity, the hydroxyl radical can react with both bases and deoxyriboses, however, as far as deoxynucleotides are concerned, the hydroxyl radical adds preferably to base moieties and the addition to deoxyribose is only about 10–15%. It may be inferred that the situation in poly G is similar.

Because the reaction of hydroxyl radicals with dGMP gives rise to oxidizing radicals (50%) and reducing radicals (50%) of dGMP-OH• [12, 37, 38], the addition of hydroxyl radical to poly G is inferred to form the same kinds of hydroxyl radical adducts with respect to redox properties as its addition to dGMP. The oxidizing radical (poly G-C4-OH•) was formed by OH• addition to C4 of the guanine moiety of poly G. This oxidizing radical should react with VER and CIS C, and therefore is likely to revert to poly G or hydrated poly G, at the same time was VER or CIS C are converted into their phenoxyl radicals (eq. 1).

$$\text{poly G-C4-OH}^{\bullet} + \text{VER (CIS C)} \rightarrow \text{poly G} + \text{VER (CIS C)-PhO}^{\bullet} \quad (1)$$

A number of investigations have shown that bases in DNA or free bases will tend to stock in aqueous solution [40]. Base stocking is favored for electron transfer between base radicals and bases, and may therefore have a negative effect on the repair of base radicals by phenolic antioxidants. However, the results of present study indicated that even of base stocking VER and CIS C still fast repair poly G hydroxyl radical adduct.

As is well known, Purine is more easily oxidized than pyrimidine by oxidants including hydroxyl radical. Therefore, in DNA hydroxyl radicals add to purine more than to pyrimidine, hence the hydroxyl radical adducts of DNA are mainly purine-type hydroxyl radical adducts. Of these hydroxyl radical adducts, the oxidizing hydroxyl radical adducts may be repaired by VER and CIS C. Our experimental results are consistent with this assumption.

The inset curve in Fig. 3A represents the change of absorption of VER-PhO• at 390 nm with time after pulse irradiation. The growth of absorbance follows first-order kinetics. The slope is the apparent rate constant (k_{app}) of generation of VER-PhO• by reaction of VER with poly G-OH•. Varying the concentration of VER (0.02–0.1 mM), a series of k_{app} are obtained. The dependence of k_{app} on [VER] is a straight line. The slope yields the rate constant (k) for electron transfer from VER to poly G-OH•. The rate constants of the fast repair reactions of poly G hydroxyl radical adduct by VER and CIS C were deduced and are shown in the Table.

Phenolic antioxidants can protect DNA from damage by reactive oxygen species (ROS) either via scavenging ROS prior to their attacking DNA or by fast repair of DNA damaged by ROS. These two ways complement each other. Our study shows that VER and CIS C are also effective hydroxyl radical scavengers, and hence protect DNA from attack by •OH [41]. The results of the present work indicated that VER and CIS C being electron donors can fast repair oxidizing hydroxyl radical adducts of poly G by electron transfer reactions. However, since the hydroxyl radical is very reactive, its life in a biological system is very short and it will react close to the site where it is generated. The protective effect exerted by scavenging hydroxyl radicals only becomes feasible at a high concentration of antioxidant. Therefore, the repair effect of the tested compounds on hydroxyl radical adducts of DNA occurs preferentially to the protection mode.

DNA lesions may become the site of a replication error and hence contribute to the pattern of mutations, or may become inhibitors of replication or transcription and hence cause a number of degenerative diseases. Although damaged DNA can be repaired by an enzymatic system, enzymatic repair of DNA damage requires a time scale of hours [42]. However, in the present study, the fast repair reaction initiated and finished in a microsecond, time scale as a result preventing this transient product from reaction with other biological macromolecules, such as protein, enzymes and so on. Therefore, the non-enzymatic repair is enormously faster by around 9 orders than enzymatic repair. Although the above mentioned fast repair was ob-

Table 1: The rate constants of repair reaction of oxidizing hydroxyl adduct of poly A by phenypropanoid glycoside

	$K/10^9\ M^{-1} \cdot s^{-1}$
Verbasicoside	1.4
Cistanoside C	1.0

served in a chemical system, in view of the existence of a series of endogenous reductants such as ascorbate, thiols etc. which can react with both dGMP-OH$^{\bullet}$ and dGMP$^{\bullet+}$ at a high rate [12] imply a potential effective repair process, it is reasonable to infer that non-enzymatic fast repair occurs also in cells. Based on this suggestion, it is proposed that non-enzymatic fast repair is restricted with regard to the primary damage induced by ROS, that is DNA radical, and that the enzymatic repair system works for the repair of steady state lesions of DNA resulting from DNA radicals. These two repair system complement each other. A deeper understanding of this new repair mechanism will undoubtedly help researchers explore new preventative and/or interventional medicine.

4. Experimental

4.1. Materials

Verbascoside (VER) and cistanoside C (CIS) were isolated and purified from *Pedicularis* species. Polyguanylic acid (poly G) was purchased from Sigma. All other chemicals were purchased from Shanghai Biochemical Corporation (Shanghai, China) and are of reagent grade.

4.2. Pulse radiolysis

Pulse radiolysis studies were conducted using a linear accelerator providing 8 MeV electron pulse with a duration of 8 ns. The dosimetery of the electron pulse was determined by a thiocyanate dosimeter containing 10 mM KSCN solution saturated with nitrous oxide, by taking $\varepsilon_{(SCN)^-} = 7600\ dm^3 \cdot mol^{-1} \cdot cm^{-1}$ at 480 nm. The detailed description of the pulse radiolysis equipment and the experimental conditions have been given elsewhere [43]. In the present work, the average pulse dose was 15 Gy.

4.3. Generation of hydroxyl radical adduct of poly G

The hydroxyl radical adduct of poly G was generated by pulse radiolysis of 4 mM poly G aqueous solutions saturated with N_2O. On pulse irradiation, hydrated electrons (e^-_{aq}), hydroxyl radicals ($^{\bullet}OH$) and hydrogen atoms (H) are produced with G's ($\mu M \cdot J^{-1}$) of 0.29, 0.29 and 0.06 through ionization of water, respectively [44]. e^-_{aq} was scavenged by saturation with N_2O to form $^{\bullet}OH$, and then $^{\bullet}OH$ reacted with DNA to generate the hydroxyl radical adduct of poly G (poly G-OH$^{\bullet}$) (eqs. (2)–(4)).

$$H_2O \rightarrow {}^{\bullet}OH + e^-_{aq} + H^{\bullet} \quad (2)$$

$$e^-_{aq} + N_2O \rightarrow {}^{\bullet}OH + OH^- + N_2 \quad (3)$$

$$\text{poly G} + {}^{\bullet}OH \rightarrow \text{poly G-OH}^{\bullet} \quad (4)$$

4.4. Fast repair system of hydroxyl radical adduct of poly G

The fast repair system was designed according to the principle of competitive reaction.

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