Protective Effects of Vitamin Glycosides on γ-radiation and H₂O₂-Induced Decomposition of Thymine in Aqueous Solutions

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Thymine/Radiolysis/Radical/Vitamine Glycosides.

Effects of ascorbic acid (AA), ascorbic acid glycoside (AAG) and α-tocopherol monoglycoside (TMG) on radiation – and H₂O₂-induced decomposition of thymine in aqueous solutions were investigated. Of the three compounds studied, AAG was found to possess the most marked protector properties. An explanation of this phenomenon has been given in terms of differences in molecular structures of AA and AAG, as well as properties of radical adducts formed during their interaction with OH radicals.

INTRODUCTION

The injuring action of ionizing radiation is due, in many respects, to reactions of water radiolysis products with DNA molecules. The main role in this process is attributed to highly reactive OH species, which interact predominantly with bases of nucleic acids.[1,2] This results in hydroxylation of purine and pyrimidine moieties. A displacement of the radical centre from the nucleobase to the carbohydrate moiety makes possible breaks in polynucleotide chains of the nucleic acid macromolecules to occur.[3]

On irradiation of cancerous cells, the above named processes lead to their death, which determines, in many respects, the necessary therapeutic effect.[4] However, the use of radiotherapy in oncology is confronted with the necessity of solving the problem of avoiding side effects due to the damage caused to healthy cells. This circumstance makes topical the search for substances that would be predominantly localized in the healthy cells, protecting them from noxious action of radiation.[5] Recent studies[6–8] have shown that ascorbic acid glycoside (AAG) and α-tocopherol monoglycoside (TMG) possess these properties. Thus, it has been demonstrated[8] that AAG produces antioxidative and radioprotective effects due to blocking the radiation-induced lipid peroxidation processes and strand breaks in DNA. It was shown that the effects of such kind are associated with OH radical scavenging by AAG molecules. At present, the studies of radical scavenging reaction of 2-substituted ascorbic acid derivatives arouse keen interest among researchers.[9,10]

In this study, we investigated the protection effect of vitamin glycosides on γ-radiation- and H₂O₂-induced decomposition of thymine (Th) in aqueous solutions. In our opinion, these data will contribute to understanding the molecular fundamentals of radioprotective action of AAG and TMG, because thymin is one of the most reactive DNA moieties with respect to OH radicals.

EXPERIMENTAL

Ascorbic acid and thymine used in this study were from Aldrich, and 30% hydrogen peroxide was from Mosreaktiv (Russia). Ascorbic acid glycoside was made available by the Health Research Foundation (Japan), and α-tocopherol monoglycoside – by the CCI Corporation (Japan). Structures of compounds used in the study are shown in Fig. 1.

The starting solutions of thymine were prepared by dissolving accurately weighed amounts in 0.1 M phosphate buffer or 10^{-3} M sodium hydrocarbonate solutions. Buffers were used to conduct experiments under identical pH values. Phosphate buffer with pH = 7,4 was used to maintain physiological conditions, NaHCO₃ was added to AAG solutions to test it's Na-salt activity.

To prepare the phosphate buffer and NaHCO₃ solutions, twice distilled water was used. Solutions containing ascorbic acid and vitamin glycosides were prepared by dissolving accurately weighed amounts of the respective compound in the starting thymine solutions. The prepared solutions were transferred into glass ampoules and sealed.
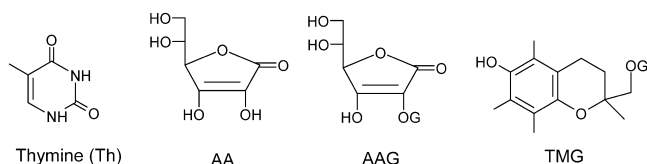
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Fig. 1. Structures of compounds used in the study (G = glucose moiety).

The ampoules with the solutions were irradiated in a γ-unit with a ^{137}Cs source. The dose rate was 0.30 Gy/s, the absorbed dose range being 0.23 to 1.00 kGy. Radiation-chemical yields of decomposition (G) were calculated from the data on thymine concentration decrease as function of dose absorbed, provided that the starting compound concentrations varied linearly within the absorbed dose range used.

To study H_2O_2-induced processes, thymine solutions (10^{-3} M) were prepared in 0.1 M phosphate buffer or 10^{-3} M sodium hydrocarbonate solutions containing 10^{-3} M H_2O_2, the exact concentration of which was determined by titration with a potassium permanganate solution. Sealed ampoules with thymine solutions containing H_2O_2, as well as AA, AAG or TMG as additives at concentrations of 10^{-3} M, were placed in a thermostat and kept at $120 \pm 1°C$ for 10–40 min. The degree of decomposition of the compounds studied was calculated in per cent of their initial concentration.
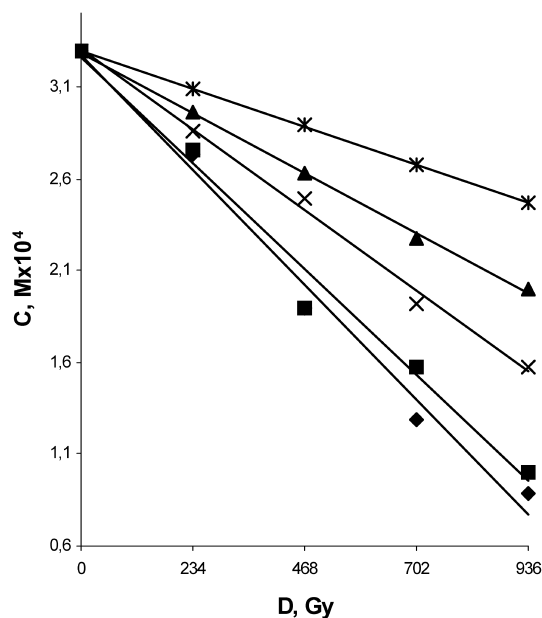
Thymine concentrations in the solutions were measured by HPLC using a LC-10 instrument (Shimadzu) equipped with a UV detector (λ 266 nm) and a 250×4 mm column packed with Nucleosil 120-5 C-18 (Macherey-Nagel). Chromatographic conditions were as follows: temperature: 20°C; eluent: 20 mM solution of NaH_2PO_4 in water/methanol mixture 90/10 v/v; solution pH: 3.0 adjusted by adding the appropriate amount of ortho-phosphoric acid; flow rate: 0.5 ml/min; injected volume: 10 μl.

RESULTS

The main purpose of the radiation-chemical experiments was finding out the effects produced by AA, AAG and TMG on thymine decomposition under γ-irradiation of its aerated solutions.

The influence of absorbed dose on changes in concentration of thymine was studied in the presence and in the absence of the test compounds, the substrate/additive ratio being 1:1. The obtained data are shown in Fig. 2.

From the kinetic data obtained (Fig. 2), radiation-chemical yields of thymine decomposition were calculated, the numeric values of which are presented in the legend to Fig. 2. These values indicate that the glycosides used (AAG and TMG) effectively protect the nucleobases of DNA from radiation injury. The most pronounced radioprotective effect was displayed by AAG, which reduced almost twice the decomposition yield for thymine on radiolysis of its buffered aque-

Fig. 2. Thymine concentration as function of absorbed dose on γ-irradiation of its $3.4 \cdot 10^{-4}$ M aqueous solutions in the presence of the test compounds at concentrations of $3.4 \cdot 10^{-4}$ M. ◆ - Th (G = 2.52 ± 0.30), ■ - Th + AA (G = 2.28 ± 0.25), ▲- Th + AAG (G = 1.35 ± 0.15), × - Th + TMG (G = 1.80 ± 0.20), ✳ - Th + AAG + NaHCO₃ (G = 0.82 ± 0.09).

ous $3.4 \cdot 10^{-4}$ M solutions, while an almost 3-fold reduction of the initial value was reached for thymine solutions containing $NaHCO_3$ in the presence of AAG.

A similar trend was observed on irradiation of aqueous thymine solutions when substantially smaller quantities of the test compounds were added (Fig. 3).

At the thymine/additive ratio of 1:0.1, the radioprotective effect was less marked. However, in this case also, the radiation-chemical decomposition yields for thymine were reduced to a substantially greater extent on addition of AAG than on addition of AA or TMG (Fig. 3).

The injury of thymine on radiolysis in diluted aqueous solutions is believed to be associated with reactions involving OH radicals. This species are also formed on thermal decomposition of hydrogen peroxide. Therefore, we investigated the influence of AA, AAG and TMG on H_2O_2-induced thymine decomposition in aqueous solutions. The obtained data on thymine decomposition (in %) are shown in Fig. 4.

As follows from the experimental results, AA enhanced the H_2O_2-induced decomposition of thymine. TMG and AAG manifested protective behaviour in this case, AAG proving to be the most efficient stabilizer of thymine under the conditions given, with no decomposition recorded in the initial period of the reaction.

We found a still more pronounced protective effect when studying thymine decomposition in the presence of AAG in aqueous solutions containing 10^{-3} M $NaHCO_3$ instead of the
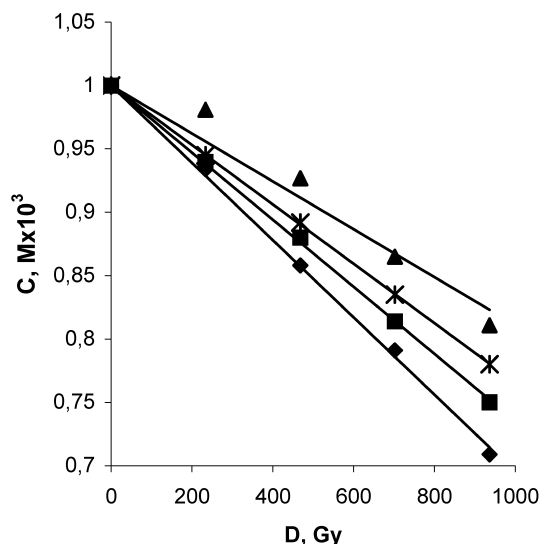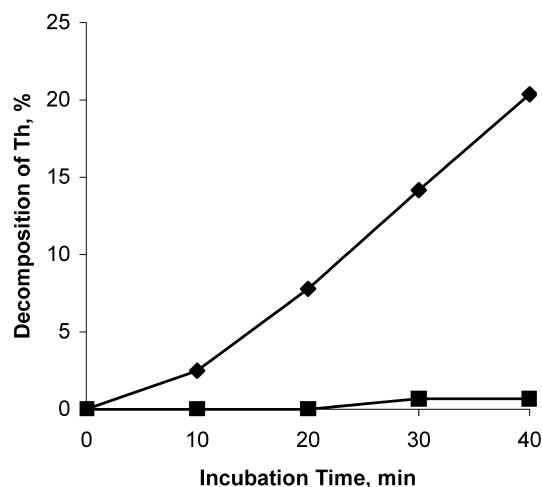
Fig. 3. Thymine concentration as function of absorbed dose on γ-irradiation of its $1 \cdot 10^{-3}$ M aqueous solutions in the presence of the test compounds at concentrations of $1 \cdot 10^{-4}$ M. ◆ - Th (G = 2.59 ± 0.15), ■ - Th + AA (G = 2.20 ± 0.20), ▲- Th + AAG (G = 2.06 ± 0.20), ✳ - Th + TMG (G = 1.70 ± 0.14).
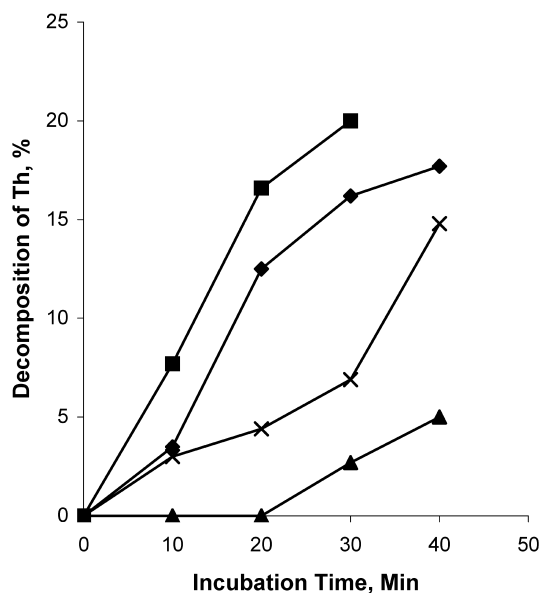
Fig. 4. Thymine decomposition (%) in its aqueous $1 \cdot 10^{-3}$ M solutions under the action of H_2O_2 (T = 120°C). ◆ - Th , ■ - Th + AA, ▲ - Th + AAG, × - Th + TMG.

Fig. 5 Thymine decomposition (%) in its aqueous $1 \cdot 10^{-3}$ M solutions under the action of H_2O_2 (T = 120°C). ◆ - Th + NaHCO₃, ■ - Th + AAG + NaHCO₃.

DISCUSSION

The data obtained in the study unambiguously indicate that AAG effectively protects thymine from injury caused by OH radicals generated by means of either water radiolysis or thermal decomposition of H_2O_2. As regards this index, AAG is superior to TMG, and especially AA. Possible causes of the observed effects may be as follows.

Earlier, it has been reliably established that the attack of OH radicals on thymin results in their addition to $> C_5 = C_6 <$ double bond, leading to formation of various mono- and di-hydroxylated derivatives of the initial compound:[3,11]

The rate constant k of reaction (1) is high: according to,[11] its value amounts to about $6.4 \cdot 10^9$ l·mol⁻¹·s⁻¹. In order to block the process (1), agents having at least equal or even higher reactivity towards OH species should be used.

AA is also known to interact effectively with hydroxyl radicals, mainly by means of addition to $> C_2 = C_3 <$ double bond. The rate constant of this reaction is $k = 1.2 \cdot 10^{10}$ l·mol⁻¹·s⁻¹.[12] Since AA is present in its dissociated state at pH values used in the study, the following reactions take place:

phosphate buffer (Fig. 5).

When AAG at concentration of 10^{-3} M was added to aqueous solutions of thymine containing NaHCO₃, the yield for thymine decomposition in the presence of H_2O_2 did not exceed 1% throughout the whole time of incubation at 120°C (Fig. 5).

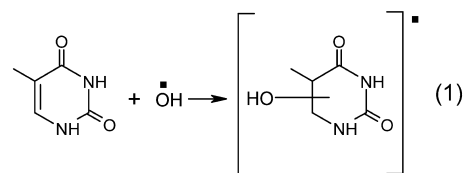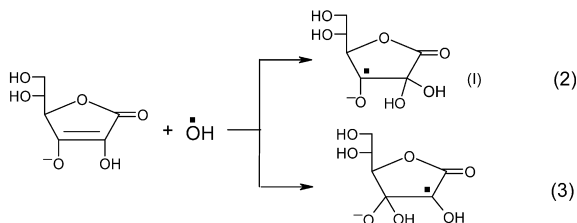AA, with its reactivity towards OH species comparable to that of thymine, when present in commensurable concentrations, should protect the initial substrate from γ- and H_2O_2-induced decomposition. However, this is not observed experimentally. Moreover, AA enhances thymine injury on thermal decomposition of H_2O_2 (Fig. 4). In our opinion, this is due to formation of additional quantities of reactive oxygen species (ROS), which can appear due to reactions of radical anions formed according to (2) with oxygen or hydrogen peroxide, i.e. the following processes take place:
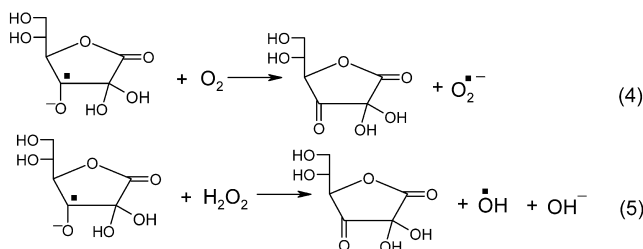
The reactions (4) and (5) are sources of additional quantities of ROS, therefore AA does not ensure an efficient protection of thymine from OH-induced injury. The possibility of realization of reactions (4) and (5) follows from the fact that radical-anions of type (I) are more effective in electron transfer reactions than AA anions.[13]

As regards the features of protective effect observed in the case of AAG, in our opinion, one should take into account the following considerations. On interaction of AAG with OH radicals, addition to $> C_2 = C_3 <$ double bonds will also take place, although reactions with the carbohydrate moieties are not excluded. In either case, the reaction rate constant for the interaction of OH with AAG will not exceed the corresponding value for AA. The addition to the $> C_2 = C_3 <$ double bond of AAG will predominantly occur at the C_3-atom, because C_2 is shielded by the glucose moiety.
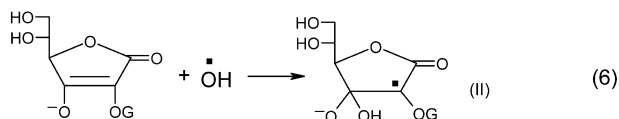
In contrast to intermediates (I) formed in the presence of AA, the radical adducts (II) resulting from reaction (6) will not interact with O_2 and H_2O_2 effectively, giving ROS. This may constitute an advantage of AGG over AA as regards radioprotective and antioxidative effects in biosystems. Apparently, the behaviour of anion-radicals of types I and II depends to a significant extent on pH of the medium, as evidenced by the data concerning effects produced by the presence of NaH_2PO_4 (pH 7.4) or $NaHCO_3$ (pH 8.5) on the observed phenomena. A possible role of the radical adducts of types (I) and (II) in manifestation of antioxidative, radioprotective or prooxidative properties by AA and its derivatives has not been discussed in the literature thus far. The details concerning effects of such type will be the investigated in further studies to be performed in our laboratory.

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