Redox Reactions of Tocopherol Monoglucoside in Aqueous Solutions: A Pulse Radiolysis Study

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The reactions between tocopherol monoglucoside (TMG), a water-soluble vitamin-E derivative, with Br₂–, N₃–, (SCN)₂–, NO₂–, OH and various halogenated peroxyl radicals were examined using a pulse radiolysis technique. The results demonstrate that TMG forms a stable phenoxy radical at pH > 6.8. The thus-formed phenoxy radical shows pH-dependent decay kinetics and is disproportionated by 2nd order kinetics at pH 2.3. It was observed that the TMG reactivity towards a halogenated peroxyl radical increases with the number of halogen atoms at the carbon atom having a peroxyl group. The reaction between the TMG phenoxy radical and ascorbic acid was also examined using a pulse radiolysis technique. The results indicate that the TMG phenoxy radical is repaired by ascorbate. Kinetic studies indicate that TMG may act as an antioxidant to repair free-radical damage to some biologically important compounds. The one-electron reduction potential for TMG was found to be 0.522 V ± 0.06 vs. NHE.

INTRODUCTION

The major free radicals produced upon the irradiation of water are H+, OH−, and eaq−. These free radicals are very reactive¹, and react with many inorganic and organic compounds, including RNA, DNA, and protein². To protect normal tissues, the role of protective compounds becomes very important. A large number of these compounds have been synthesized. Unfortunately, most of them have been found to be toxic. Recently, promising results have been shown for WR 2721 and other protectors in its series³–⁸. The important feature of these radio protectors is that they are water soluble, which facilitates administration. Also, reactive oxygen species, derived from molecular oxygen, have been known for their deleterious action⁹–¹¹. Vitamin-E (of which α-tocopherol is the major component) is present in cells and has been shown to be an inhibitor of free radical-mediated membrane damage¹². It acts as a free-radical scavenging antioxidant by scavenging peroxyl radicals.
radical reactions, and other related reactions and it is suggested that it may prevent cancer. Recently, it has been suggested that the presence of selenium improves the immune-boosting activity, and hence it defends out bodies against free radicals\textsuperscript{13}. It works best along with β-carotene and Vitamin-E. The activity of all these antioxidants together takes the immune function to a higher level. Because Vitamins A and E are lipophilic, their local concentrations in specific cellular compartments are generally high enough for a protective effect. This is in contrast to most water-soluble sulphhydryl compounds\textsuperscript{13}. Because α-tocopherol is soluble in organic solvents and membranes, most studies have been conducted in organic and liposome systems. In aqueous solution, Trolox-C, a water-soluble phenolic antioxidant in which the polyisoprenoid tail of α-tocopherol is replaced by a carbon moiety, is used to carry out redox reactions\textsuperscript{14,15}. To mimic Vitamin-E more closely, in the present study we used a water-soluble Vitamin-E derivative, tocopherol monoglucoside (TMG), Scheme 1. It was shown that TMG is very effective in protecting DNA against γ-irradiation\textsuperscript{16–20}. In the present paper we describe studies on the oxidation of TMG by various free radicals.

\begin{center}
\textbf{Tocopherol monoglucoside}
\end{center}

\begin{center}
\textbf{Scheme 1}
\end{center}

\textbf{MATERIALS AND METHODS}

TMG was obtained from Dr. V.T. Kagiya, Health Research Foundation, Kyoto, Japan, and used as received. All other chemicals and reagents were of HPLC, AR or GR grade. IOLAR grade (purity > 99.9%) gases (N\textsubscript{2} or N\textsubscript{2}O) used for purging the solutions were obtained from Indian Oxygen Limited.

All solutions were prepared in a 10\textsuperscript{−3} mol dm\textsuperscript{−3} phosphate buffer (equimolar mixtures of Na\textsubscript{2}HPO\textsubscript{4}, H\textsubscript{2}O and KH\textsubscript{2}PO\textsubscript{4}) unless otherwise stated. The acidic and alkaline pH was adjusted by KH\textsubscript{2}PO\textsubscript{4}, HClO\textsubscript{4} and NaOH to the required value. Nanopure water (conductivity of 0.1 \(\mu S\) cm\textsuperscript{−1}) from a Barnstead (USA) nanopure water unit was used for making solutions.

\textbf{Radiolytic Procedures}

Pulse radiolytic studies were carried out by irradiating solutions in rectangular quartz cells of 1 cm optical path length. Pulses of 50 ns, 500 ns, and 2 \(\mu S\) of 7 MeV electrons from a linear electron accelerator were employed. The details of the LINAC are given elsewhere\textsuperscript{21}. An aerated 5 \(\times 10^{-2}\) mol dm\textsuperscript{−3} KSCN solution was used for dosimetry and the (SCN)\textsubscript{2}•− radical was monitored at 500 nm. The absorbed dose per pulse was calculated assuming Ge for the (SCN)\textsubscript{2}•− radical to be 21,520 dm\textsuperscript{3} mol\textsuperscript{−1} cm\textsuperscript{−1} per 100 eV\textsuperscript{22}, where G is the radiation chemical yield expressed as the number of molecules formed or destroyed per 100 eV of energy absorbed, and \(\varepsilon\) is the molar absorptivity. The dose employed in the present study, unless otherwise stated, was typically 16 Gy per pulse. However, a lower dose of about 9 Gy per pulse was used to determine the formation rate constants, the molar absorptivity and the equilibrium constant.

\textbf{Reactions of TMG with OH radical and one-electron oxidants}

Upon irradiating H\textsubscript{2}O, the following primary radicals are produced\textsuperscript{1}:

\begin{equation}
\text{H}_2\text{O} \rightarrow e_{\text{aq}}\textsuperscript{−}, \cdot\text{H}^\cdot, \cdot\text{OH}\cdot.
\end{equation}
In order to selectively produce OH radicals, solutions were saturated with N$_2$O prior to pulse irradiation. This resulted in the scavenging of e$_{aq}^-$, under these conditions the yield$^1$ of the OH radicals was 90% of the total G(OH$^\cdot$) = 5.4 mol/100 eV = 5.6 × 10$^{-7}$ mol J$^{-1}$. The remaining 10% contribution was of H$^\cdot$ atoms, with G(H$^\cdot$) = 0.6 mol/100 eV = 0.62 × 10$^{-7}$ mol J$^{-1}$.

\[ e_{aq}^- + N_2O \rightarrow H_2O \rightarrow N_2 + OH^- OH^\cdot \]  
(2)

Secondary one-electron oxidants were produced by the reaction of OH$^\cdot$ radicals with inorganic anions$^23$ via the following reactions:

\[ OH^\cdot + N_3^- \rightarrow N_3^- + OH^- \]  
(3)

\[ OH^\cdot + Br^- \rightarrow Br^- + OH^- \]  
(4)

\[ Br^- + Br^- \rightarrow Br_2^- \]  
(5)

\[ OH^\cdot + NO_2^- \rightarrow NO_2^- + OH^- \]  
(6)

Subsequent reactions of the secondary oxidants, (N$_3^\cdot$, Br$_2^-\cdot$ and NO$_2^-\cdot$) with TMG resulted in the generation of a one-electron oxidised species of TMG.

Reactions of peroxyl radicals

Peroxyl radical reactions were studied in aerated alcohol or alcohol-water solutions. They were produced in solution by de-halogenation from the respective molecule, as explained in the literature$^{24-26}$. The rates of the reactions were determined by carrying out the experiments with at least three different concentrations of TMG, which varied by at least a factor of 4. The increase in the pseudo-first-order rate for the formation of the phenoxy radical with the concentration of TMG was calculated at 430 nm. The bimolecular rate constants were derived from plots of the first-order rates vs. concentration. The rate constants reported in the table are generally accurate to ± 15%. The second-order constants were checked at different doses (16 Gy – 80 Gy).

Cyclic voltammetry experiments were carried out employing an Autolab Electrochemical system (Eco Chemie, Netherlands) equipped with a PGSTAT-100 and driven by software, GPES. The electrochemical system was coupled to a cell comprising a Pt disc as a working electrode (3mm diameter), a saturated calomel reference electrode (SCE) and a Pt rod as a counter electrode. The experiments were performed in a N$_2$- bubbled aqueous solution at 25°C. The values of the potentials obtained vs. SCE were converted to a normal hydrogen electrode (NHE) by adding 0.24 V.

RESULTS AND DISCUSSION

Reactions of inorganic radicals with TMG

Figure 1 shows the spectra of the transient obtained in a N$_2$O saturated solution containing 2 × 10$^{-4}$ mol dm$^{-3}$ TMG and 10$^{-1}$ mol dm$^{-3}$ KBr at various pH values. It can be seen that the transient shows...
the absorption maximum at wavelengths of 320 and 430 nm. The observed spectrum is similar to that previously assigned to the Trolox C phenoxyl radical\(^{14,15}\). It can also be seen that the transient yield is not similar at different pH values. It has been suggested that G(Br\(_2^–\)) varies with the pH\(^{15}\). Therefore, the above variation in the yields of phenoxyl radical may be due to this effect. The rate constant for the formation of the phenoxyl radical was found to be independent of the pH (2.3–11.2). It was observed that the phenoxyl radical followed a pH-dependent 2\(_{nd}\) order decay. Above pH 6.8 (up to 11.2) no decay of the phenoxyl radical was observed under our experimental time window. However, at pH 2.3, it decayed by the 2\(_{nd}\) order rate with \(2k/\epsilon l = 9.8 \times 10^4\) s\(^{–1}\). Figure 2 shows the time-resolved absorption spectrum of the intermediate formed due to the decay of the phenoxyl radical at pH 2.3. It can be seen that with time the band at 330 nm develops with a concomitant increase in the absorption at a wavelength below 310 nm. It is known that the phenoxyl radical upon decay forms a cross-conjugated ketone, which shows an absorption band in the wavelength region below 350 nm\(^{15}\). Therefore, it appears that the observed absorption band in Fig. 2 at 1600 \(\mu\)s could be due to the formation of a cross-conjugated ketone.

Upon pulse radiolysis of a \(\mathrm{N}_2\mathrm{O}\) saturated solution containing \(10^{-1}\) mol dm\(^{-3}\) azide and \(10^{-4}\) mol dm\(^{-3}\) TMG, a transient similar to that observed with Br\(_2^–\) was formed. The molar absorptivity of the phenoxyl radical was found to be \(6,500\) dm\(^{3}\) mol\(^{-1}\) cm\(^{-1}\). This value is in reasonable agreement with the reported value\(^{14}\) of \(7,100\) dm\(^{3}\) mol\(^{-1}\) cm\(^{-1}\). A similar

![Fig. 2](image_url)

**Fig. 2.** Time-resolved transient absorption spectra obtained after the pulse radiolysis of a \(\mathrm{N}_2\mathrm{O}\) - saturated aqueous solution containing \(2 \times 10^{-4}\) mol dm\(^{-3}\) TMG and \(10^{-1}\) mol dm\(^{-3}\) KBr at pH’s 2.3, \(\square\) 50 \(\mu\)s, \(\square\) 200 \(\mu\)s, \(\square\) 600 \(\mu\)s, \(\square\) 1600 \(\mu\)s.

### Table 1. Spectral and kinetic parameters of the transients formed by the reaction of TMG with various radicals

| Radical          | pH  | \(k, \text{dm}^3\text{mol}^{-1}\text{s}^{-1}\) |
|------------------|-----|---------------------------------|
| \(\text{Br}_2^*\) | 2.3 | \(1.0 \pm 0.2 \times 10^9\)      |
| \(\text{Br}_2^*\) | 7   | \(1.0 \pm 0.2 \times 10^9\)      |
| \(\text{Br}_2^*\) | 11.2| \(1.0 \pm 0.2 \times 10^9\)      |
| \(\text{OH}^*\)  | 7   | \(3.7 \times 10^9\)             |
| \(\text{OH}^*\)  | 11  | \(3.7 \times 10^9\)             |
| \(\text{N}_3^*\) | 7   | \(4.0 \times 10^9\)             |
| \((\text{SCN})_2^*\) | 7 | \(1.1 \times 10^9\)            |
| \((\text{SCN})_2^*\) | 12 | \(1.2 \times 10^9\)            |
| \(\text{NO}_2^*\) | 8.4 | \(0.6 \times 10^8\)            |
| \(\text{NO}_2^*\) | 11.8| \(8.0 \times 10^8\)            |
| \(\text{CBr}_3\text{O}_2^*\) | 7 | \(6.6 \times 10^8\)            |
| \(\text{CCl}_3\text{O}_2^*\) | 7 | \(4.1 \times 10^8\)            |
| \(\text{CHB}_r\text{O}_2^*\) | 7 | \(1.1 \times 10^8\)            |
| \(\text{CHCl}_2\text{O}_2^*\) | 7 | \(7.7 \times 10^7\)            |
| \(\text{CH}_3\text{ClO}_2^*\) | 7 | \(3.0 \times 10^7\)            |
| \(\text{CHCl}_3\text{O}_2^*\) | 7 | \(2.0 \times 10^7\)            |
| \(\text{CH}_3\text{ClO}_2^*\) | 7 | \(3.8 \times 10^7\)            |
| \(\text{ClCH}_2\text{CCl}_2\text{O}_2^*\) | 7 | \(3.6 \times 10^7\)            |
result was obtained when the thiocyanate radical anion (SCN)\(_2\)\(^-\) was used as an oxidant. The bimolecular rate constants calculated for the reactions of various inorganic radicals with TMG are listed in Table 1.

Reactions of the hydroxyl radical with TMG

Figure 3 shows the absorption spectrum of the transient obtained upon the pulse radiolysis of an aqueous \(\text{N}_2\text{O}\) saturated solution of TMG (2 \(\times\) 10\(^{-4}\) mol dm\(^{-3}\)) at pH 6.8 (dose = 16 Gy). The transient shows an absorption maximum at 430 nm. The obtained spectrum is similar to that observed with \(\text{Br}_2\)\(^-\) (Fig. 1). Similar results were obtained when the experiment was carried out at pH 11. It can be seen from Fig. 3 that the decrease in the absorbance of the transient was faster at 320 nm compared to that at 430 nm. Also, the band at 320 nm became broader with a slight shift towards a higher wavelength. In the case of TMG, there are several sites available for an attack of the OH\(^-\) radical. Because the OH\(^-\) radical can abstract hydrogen from the phenolic group, it can add to the phenolic ring and can also form an adduct or abstract hydrogen from the glucose moiety. All of these processes can take place in parallel. In addition, there is a possibility of an intramolecular process in which the radical centre from the glucose moiety can become transferred to TMG. It appears that the initial absorption at 320 nm is due to an OH adduct. This is consistent with studies reported for phenol, where, it has been shown that the initial radical is the formation of an OH-adduct, which absorbs at 320 nm. It subsequently eliminates water in an acid/base catalysed reaction\(^{27}\). The observed shift in the absorption maximum at wavelengths less than 350 nm is probably due to the above-mentioned reaction. The formation rate of the transient at 430 nm was observed to increase with the concentration of TMG. The bimolecular rate constant was obtained from the slope of the pseudo-first-order rate constant with respect to the TMG concentration. The overall bimolecular rate for the formation of the phenoxy radical was found to be 3.7 \(\times\) 10\(^9\) dm\(^3\) mol\(^{-1}\) s\(^{-1}\). The results are compiled in Table 1.

Reactions with NO\(_2\)\(^-\) and haloperoxyl radicals

The oxides of nitrogen are stable radicals, and are a matter of concern due to vehicular and industrial pollution. In particular, NO\(_2\)\(^-\) is known to abstract H-atoms from unsaturated lipids. It thus acts as an initiator of lipid per oxidation. Similarly, halogenated organic compounds are widely used as solvents, pesticides and refrigerants. Their environmental impact is being viewed with serious concern. Many of these are also toxic to living systems\(^{28}\). It is known that in living systems these compounds undergo one-electron reduction to loose a halide ion. On subsequent reaction with an oxygen peroxyl radical\(^{24-26}\) is formed. These peroxyl radicals lead to lipid per-oxidation \textit{in vivo} as well as \textit{in vitro}. Therefore, an attempt has been made to study the reactions of various halogenated peroxyl and NO\(_2\)\(^-\) radicals with TMG.

The nitrogen dioxide radical, NO\(_2\)\(^-\), was pre-
pared by a reaction of OH• with NaNO2 in a N2O-saturated aqueous solution containing 10⁻¹ mol dm⁻³ NaNO2. It was observed that it undergoes a one-electron transfer reaction with TMG in an aqueous solution. The reaction of NO2• with TMG was found to be dependent on the pH. The results are reported in Table 1.

Upon a pulse radiolysis of an air-saturated aqueous solution containing 5 mol dm⁻³ 2-propanol, a 10⁻² mol dm⁻³ halogenated compound and 10⁻⁴ mol dm⁻³ TMG, the absorption spectrum of the TMG phenoxyl radical was observed. The bimolecular rate constants for the reaction of various halogenated peroxyl radicals with TMG are compiled in Table 1. It can be seen that the derived rate constants decrease upon decreasing halo substitution on the carbon-containing peroxyl group.

Regeneration of TMG

In vivo, the role of ascorbic acid in repairing various biologically important compounds is also well known. The regeneration of a vitamin through repair of the radical could result in a greater antioxidant efficiency. Such a synergistic process between vitamin E and vitamin C has been shown. To study the reactions between TMG and ascorbic acid, the following experiment was carried out. For the pulse irradiation of an aqueous solution containing 2.0 × 10⁻³ mol dm⁻³ TMG, 1.0 × 10⁻³ ascorbate and 10⁻¹ mol dm⁻³ NaN₃ at pH 7, the transient absorption spectrum obtained at different time scales is shown in Fig. 4. It can be seen from this figure that as the absorption at 435 nm decreased and there was a concomitant increase in the absorption at 360 nm. Both the increase in the formation at 360 nm and the decay at 435 nm were exponential and 1st order with respect to ascorbate concentration.

\[
\text{TMG}^\bullet + \text{Asc}^- \rightarrow \text{TMG}^+ + \text{Asc}^{\cdot-}
\]  

(7)

The bimolecular rate constant for the reaction of ascorbate with the TMG• radical, calculated by plotting the observed pseudo first-order-rate constant against ascorbate concentration, was found to be 3.8 × 10⁷ dm⁻³ mol⁻¹ s⁻¹. The above results show that reaction (7) is thermodynamically favourable only when the redox potential of TMG/TMG• is lower than Asc•⁻/AscH⁻. Because TMG is sparingly soluble in water, this may have important biological implications if TMG is used as a radio protector.

The results presented here demonstrate that the TMG phenoxyl radical shows similar characteristics to that of Trolox-C. It has been suggested that Trolox and its derivatives, which have a hydroxy methyl or carboxyl moieties at the 2-position, are not good models for comparing the behaviour of α-tocopherol radicals in the absence of oxygen. In TMG, compared to Trolox-C, at the 2-position neither hydroxy methyl nor the carboxyl moieties are there; therefore, it appears that TMG may serve as a good model for comparing reactions of α-tocopherol.
One-electron reduction potential

Determining the reduction potential of an antioxidant/radio protector is very important to obtain information about the ease with which it can undergo an electron-transfer reaction with an oxidizing radical. The reduction potential of TMG was determined by cyclic voltammetry (cv) of a deaerated aqueous solution containing $10^{-1}$ mol dm$^{-3}$ KCl, $1.0 \times 10^{-3}$ mol dm$^{-3}$ phosphate buffer and $1.0 \times 10^{-3}$ mol dm$^{-3}$ TMG at a scan rate of 50 mV/s in the 0–0.7 V range. The results are shown in Fig. 5 a. Only one oxidation peak was observed in the forward scan along with a shoulder. To resolve the observed shoulder, differential pulse voltammetry (dpv) was performed which clearly showed two peaks (Fig. 5b). This indicates that there are two oxidation sites in TMG. The probable sites are phenoxyl and the glucose moiety in the TMG. In a reverse scan of cv, one peak was observed. This indicated that the oxidation products were stable. The reduction potential was determined from the maximum potential value ($E_p$), and was found to be $E_{1\gamma} = 0.522 \pm 0.06$ vs NHE.

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