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Evidence for a possible role of 3-hydroxyanthranilic acid as an antioxidant

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The reactions of 3-hydroxyanthranilic acid (3-OHAA) with $N_3^\bullet$, $NO_2^\bullet$, $NO^\bullet$, $CCl_3O_2^\bullet$, and $OH^\bullet$ radicals were examined using a pulse radiolysis technique mainly at pH 7. The bimolecular electron transfer from secondary one-electron oxidants results in the formation of anilino radical ($\lambda_{max} \cong 380$ nm). The rate constant for the reaction of $N_3^\bullet$ radical with 3-OHAA at pH 7 was found to be 6.3×10^9 dm^3 mol^{-1} s^{-1}. It was observed that the 3-OHAA reacts with oxygen centered radicals. The repair rate constant for the electron transfer reaction from 3-OHAA to guanosine radical and chlorpromazine cation radical was also examined using a pulse radiolysis technique. Kinetic studies indicate that 3-OHAA may act as an antioxidant to repair free-radical damage to above mentioned biologically important compounds. The rate constants of electron transfer from the 3-OHAA to the guanosine and chlorpromazine radicals were determined. The one-electron reduction potential for 3-OHAA radical was found to be 0.53 ± 0.06 V *versus* NHE.

Supporting information may be found in the online version of this article.

Keywords: pulse radiolysis; electron transfer; reduction potential; free radical

INTRODUCTION

Phenolic and polyphenolic compounds including flavonoids derived from herbs, flowers, fruits, and berries, are known to possess diverse biological activity ranging from radical scavenging, anti-bacterial and anti-viral properties.[1–3] A large amount of data exists on both *in vitro* as well as *in vivo* studies carried out in assessing some of these properties. There is now enough evidence which suggests that an increased in-take of antioxidant rich fruits and vegetables, decreases the risk of developing or contacting many chronic conditions.[4–6]

3-Hydroxyanthranilic acid (3-OHAA) on the other hand, is one of the metabolites of tryptophan produced *in vivo* along the metabolic route known as the kynurenine pathway during inflammation or infection. At physiological pH it exists in de-protonated form (Scheme 1). Numerous biological studies on 3-OHAA are reported in literature.[7–9]

Aromatic amines and phenols are often used as antioxidants.[8,10–18] Their antioxidant action stems from the fact that N—H and O—H bonds are relatively weaker than the C—H bond. Hence, these compounds act as H atom donors to scavenge free radicals. In the case of aromatic amines, an understanding of the ionization behavior is difficult due to the fact that they have low ionization potentials (I.g ~ 7.5 eV) and high reduction potential ($E^o = 1$ V *vs.* NHE). Hence, studies with these types of compounds were done with a view to elucidate the ionization mechanism in the liquid phase.[10–14] To study these processes, time resolved kinetic techniques often being employed for characterizing the transients. Recently, Brede *et al.*[13,14,17] have done exhaustive studies on the ionization of the aromatic amines in non-polar solvents to correlate the influence of structure on the reaction mechanistic paths. It is suggested that in the case of aniline and phenols, free electron transfer (FET) leads to the formation of radical cation and neutral (anilino or phenoxyl) radical in equal ratio.

3-OHAA contains both phenolic and amino groups. Since its antioxidant properties have not been fully assessed, it becomes important to study the reactivity and scavenging of various ROS/RNS radicals that are generated in human beings under oxidative stress. Thus, the aim of the present work presented in this paper was to study the free radicals reactivity toward 3-OHAA. In addition, repair of various biologically important model compounds by 3-OHAA was studied. Results were elucidated by comparing results with anthranilic acid where the phenolic group is not present.

EXPERIMENTAL

Chemicals

3-OHAA and anthranilic acid were obtained from Sigma and used without further purification. All other chemicals and reagents were of HPLC, AR, or GR grade. IOLAR grade (purity > 99.9%) gases (N_2 or N_2O) used for purging the solutions were obtained from Indian Oxygen Limited.

All sample solutions were freshly prepared in Milli-Q filtered (Millipore) water and contained (10^{-3} mol dm^{-3}) phosphate buffer. The pH of the solution was adjusted by using either

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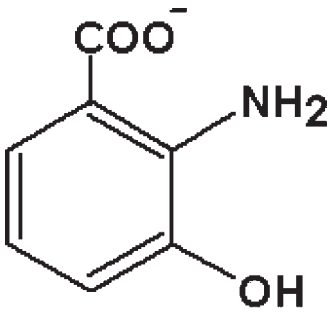

Scheme 1.

phosphate buffer or NaOH and was subsequently saturated with either N_2O or N_2 gases prior to irradiation.

Electron pulse radiolysis

For pulse radiolysis studies, a 7 MeV linear electron accelerator delivering pulses of 0.5–2 μs duration were used. The details of the LINAC are given elsewhere.[19] An aerated 5×10^{-2} mol dm^{-3} KSCN solution was used for dosimetry and the $(SCN)_2^{\bullet-}$ radical was monitored at 500 nm. The absorbed dose per pulse was calculated assuming $G\varepsilon$ for the $(SCN)_2^{\bullet-}$ radical to be 21 520 dm^3 mol^{-1} cm^{-1} per 100 eV[20] where, G is the radiation chemical yield expressed as the number of molecules formed or destroyed per 100 eV of energy absorbed and ε is the molar absorptivity. The dose employed in the present study unless otherwise stated was typically 16 Gy per pulse.

Radiolysis of water generates e_{aq}^-, $^\bullet$OH, and H$^\bullet$ as primary radical species (reaction 1) with G values of 0.28, 0.28, and 0.06 μmol J^{-1} respectively.[21,22] Besides, a small yield of molecular products such as H_2O_2 and H_2 are also formed. The yield of $^\bullet$OH radicals can be doubled by bubbling N_2O gas through water prior to radiolysis as the e_{aq}^- is quantitatively converted to $^\bullet$OH radical (reaction 2). The $^\bullet$OH radical is not a pure one-electron oxidant, as it also undergoes addition to the phenolic ring. However, it can be converted to other one-electron oxidants[23,24] (*viz.*, $N_3^\bullet$, $NO_2^\bullet$, NO$^\bullet$, and $CCl_3OO^\bullet$) through reactions 3–10 by standard methods.

$$H_2O \rightsquigarrow {}^\bullet OH,\ H^\bullet,\ e_{aq}^-,\ H_2O_2,\ H_2,\ H_3O^+ \quad (1)$$

$$e_{aq}^- + N_2O \xrightarrow{+H_2O} N_2 + {}^\bullet OH + OH^- \quad (2)$$

$$^\bullet OH + N_3^- \rightarrow N_3^\bullet + OH^- \quad (3)$$

($E_7 = 1.32$ V *vs.* NHE)[25]

$$^\bullet OH + NO_2^- \rightarrow NO_2^\bullet + OH^- \quad (4)$$

($E_7 = 1.03$ V *vs.* NHE)[26]

$$e_{aq}^- + NO_2^- \rightarrow NO_2^{\bullet-} \xrightarrow{+H^+} NO^\bullet + OH^- \quad (5)$$

($k = 4.3 \times 10^4$ dm^3 mol^{-1} s^{-1})[27]

$$^\bullet OH + (CH_3)_2CHOH \rightarrow (CH_3)_2^\bullet COH + H_2O \quad (6)$$

$$H^\bullet + (CH_3)_2CHOH \rightarrow (CH_3)_2^\bullet COH + H_2 \quad (7)$$

$$e_{aq}^- + CCl_4 \rightarrow {}^\bullet CCl_3 + Cl^- \quad (8)$$

$$CCl_4 + (CH_3)_2^\bullet COH \rightarrow {}^\bullet CCl_3 + (CH_3)_2CO + HCl \quad (9)$$

$$^\bullet CCl_3 + O_2 \rightarrow CCl_3OO^\bullet \quad (10)$$

($E_7 = 1.44$ V vs NHE)[23]

Cyclic voltammetry

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 glassy carbon as a working electrode (3 mm 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 *versus* SCE were converted to a normal hydrogen electrode (NHE) by adding 0.24 V to the experimental values.

RESULTS AND DISCUSSION

Reactions of 3-OHAA with $^\bullet$OH and $N_3^\bullet$ radical

The transient spectrum observed in the pulse radiolysis of a N_2O-saturated solution containing (5×10^{-4} mol dm^{-3}) 3-OHAA and (10^{-3} mol dm^{-3}) phosphate buffer at pH 7 shows absorption maximum at ~380 nm and broad absorption at wavelengths higher than 475 nm (Fig. 1). The rate of formation of the transient at 380 nm increased with increase in the concentration of 3-OHAA. However, the increase was not observed to vary exponentially with 3-OHAA concentration. It is known that $^\bullet$OH radical reacts with substituted benzene predominantly by addition to the ring and not by interaction with the substituent. The pattern of $^\bullet$OH radical attachment to the ring positions is expected to depend on the electron donating/withdrawing properties of the substituent. The reaction of $^\bullet$OH radical with them proceeds by addition to yield (isomeric) dihydroxy and (isomeric) hydroxy cyclohexadienyl radicals, respectively, which show absorption in the wavelength region less than 360 nm.[28] It is also reported that $^\bullet$OH radical on reaction with aniline leads to the formation of anilino radical which shows absorption maximum at *ca.* 400 nm.[10–12] Thus, the overlapping of the bands of cyclohexadienyl and anilino radical

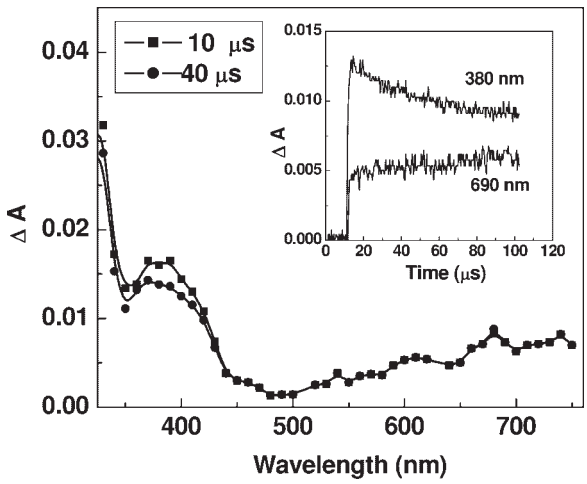

Figure 1. The transient spectrum formed by the reaction of $^\bullet$OH radical with 3-OHAA on pulse irradiation of a N_2O-saturated solution containing (5.0×10^{-4} mol dm^{-3}) 3-OHAA and (1.0×10^{-3} mol dm^{-3}) phosphate buffer at pH 7: (!) 10 μs and (,) 40 μs after the 500 ns electron pulse. Dose = 16 Gy/pulse. Inset: Decay traces at different wavelengths (i) 380 nm and (ii) 690 nm

could be the reason for not observing the exponential increase in the rate of formation. This is also clear from the time profiles of the transients at 380 and 690 nm (inset Fig. 1). Nevertheless, taking into consideration the work reported by Solar *et al.*[29] for tyrosine where it is shown that the ortho adduct of $^{\bullet}OH$ radical with tyrosine decays by H_2O elimination leading to the formation of tyrosyl radicals. It is presumed that phenoxyl radical also contributes in the overlapping of bands in addition to the cyclohexadienyl and aniline radical. It is pertinent to mention here that due to the overlapping of transient bands, the bimolecular rate constant for the reaction of $^{\bullet}OH$ radical with 3-OHAA was not determined.

Since the $^{\bullet}OH$ radical reacts with aromatic substrates by one-electron transfer, by addition to aromatic ring, or by abstraction of hydrogen, we have used azide radical to generate pure semi-oxidized species of 3-OHAA. Figure 2 shows the spectrum of the semi-oxidized species of 3-OHAA formed on pulse radiolysis of a N_2O-saturated solution containing (10^{-2} mol dm^{-3}) NaN_3, (2×10^{-4} mol dm^{-3}) 3-OHAA, and (10^{-3} mol dm^{-3}) phosphate buffer at pH 7. The kinetics of build-up of the transient at 400 and at 690 nm as a function of solute concentration was found to be 6.3×10^9 dm^3 mol^{-1} s^{-1} at both the wavelengths. As 3-OHAA contains both —NH_2 and —OH groups, $N_3^{\bullet}$ radical can react with both the groups. It is known that one-electron oxidation of aniline leads to the formation of anilino radical which shows absorption maximum at *ca.* 400 nm.[10–12,30] The bimolecular rate constant for the reaction of $N_3^{\bullet}$ radical with aniline and phenol at pH 5.8 is reported to be 4.2×10^9 dm^3 mol^{-1} s^{-1} and 5.0×10^7 dm^3 mol^{-1} s^{-1}, respectively.[30] The weak absorption band at around 700 nm was also observed in earlier study when an aqueous solution of 2-amino phenol was pulse irradiated. This band was assigned to the anilino radical.[31]

On comparing the results obtained in Figs. 1 and 2, it can be seen that the features of absorption spectrum is almost similar. However, the absorption yield at *ca.* 400 nm is less in case of $N_3^{\bullet}$ radical spectrum. This is surprising taking into consideration the fact that the yield of $^{\bullet}OH$ radical and $N_3^{\bullet}$ radical remain similar under the experimental conditions used. The only plausible reason for this difference could be due to the overlapping of phenoxyl and cyclohexadienyl type of radical with anilino radical in the case of $^{\bullet}OH$ radical reaction. It is further evident on comparing the decay traces of the transients obtained at 380 and 690 nm (insets Figs. 1 and 2).

In literature there are several reports on the oxidation of phenols.[15,16] It is known that phenoxyl radical shows absorption maximum at 410 nm. Oxidation of aromatic amines leads to the formation of the transients that shows absorption maximum at around 400 nm.[13,14,17] In a previous pulse radiolysis study on aniline in aqueous medium,[10–12] it was shown that the transient formed at ~300 and 400 nm and it is attributed to the formation of anilino radical. More recently, Maroz *et al.*[13] have carried out pulse radiolysis of aniline in *n*-butyl chloride and have concluded that the transient formed at ~415 nm is due to the formation of anilino radical whereas, the aniline cation radical shows peak at 430 nm. The aniline cation radical is too short lived due to the fast deprotonation and hence not observed in aqueous medium.[10–12] In the case of indoles the rate of reaction was 1.6×10^{10} dm^3 mol^{-1} s^{-1}.[32] Phenolic cation radical is observed in only highly acidic solutions.[23] Thus, on comparing the transient absorption bands in aqueous solution obtained in Fig. 2 with that reported in literature for aniline[11,12,15] and 2-aminophenol,[31] the transient band having $\lambda_{max} \sim 400$ nm and at wavelengths higher than 500 nm are assigned to anilino radical.

To further substantiate our conclusions regarding the reaction of $^{\bullet}OH$ and $N_3^{\bullet}$ radicals with 3-OHAA, we have carried out $^{\bullet}OH$ and $N_3^{\bullet}$ radical reactions with anthranilic acid using pulse radiolysis technique. This compound is similar to 3-OHAA but does not contain hydroxyl group. The transient absorption spectrum obtained on pulse irradiation of N_2O saturated aqueous solution containing (10^{-3} mol dm^{-3}) phosphate buffer, (10^{-2} mol dm^{-3}) NaN_3, and (5×10^{-4} mol dm^{-3}) anthranilic acid is shown in Fig. 3. It can be seen that the obtained absorption spectrum shows only one absorption maximum at *ca.* 400 nm and a broad absorption at wavelengths higher than 500 nm. The obtained spectrum agrees well with the reported spectra for aniline radical.[10–12]

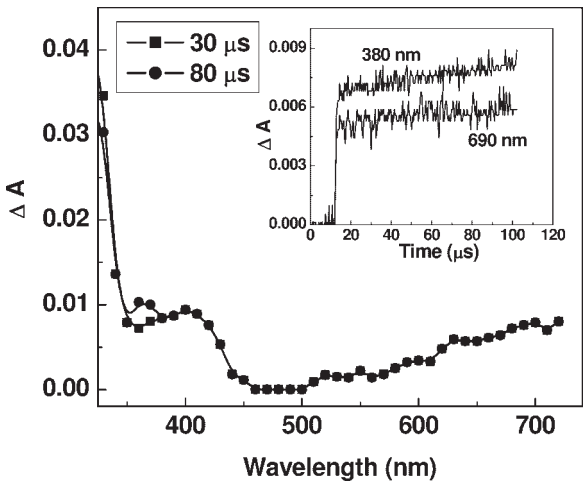

Figure 2. Transient time-resolved spectra of the semi-oxidized species of 3-OHAA formed on pulsing a N_2O-saturated solution containing (1.0×10^{-2} mol dm^{-3}) NaN_3, (2.0×10^{-4} mol dm^{-3}) 3-OHAA, and (1.0×10^{-3} mol dm^{-3}) phosphate buffer at pH 7: (!) 30 μs and (,) 80 μs after the 500 ns electron pulse. Dose = 16 Gy/pulse. Inset: Decay traces at different wavelengths (i) 380 nm and (ii) 690 nm

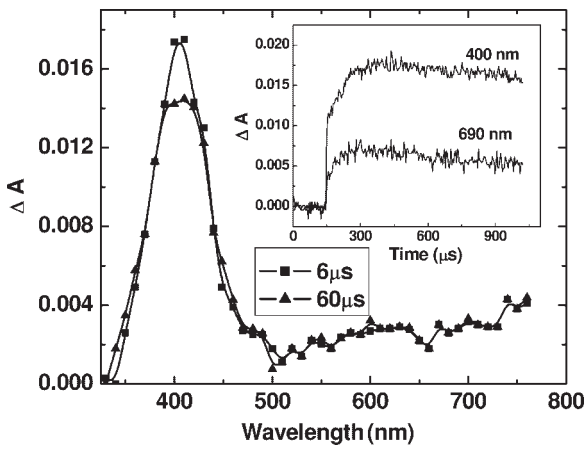

Figure 3. Transient time-resolved spectra of the semi-oxidized species formed by the reaction of azide radicals with anthranilic acid at (!) 6 μs and (*7*) 60 μs after the 500 ns electron pulse. Matrix: N_2O-saturated aqueous solution containing (10^{-2} mol dm^{-3}) of sodium azide and (5.0×10^{-4} mol dm^{-3}) of anthranilic acid at pH = 7. (Dose 16 Gy/pulse) Inset: Decay traces at different wavelengths (i) 400 nm and (ii) 690 nm

Table 1. Reaction of various oxidizing and reducing species with 3-hydroxyanthranilic acid and anthranilic acid in aqueous solution

Reaction	pH	Wavelength (nm)	Rate constant ($dm^3\,mol^{-1}\,s^{-1}$)
$N_3^\bullet$ + 3-OHAA	7	400	6.3×10^9
		690	6.3×10^9
$N_3^\bullet$ + anthranilic acid	7	400	7.2×10^9
$CCl_3OO^\bullet$ + 3-OHAA	7	410	0.6×10^8
$NO_2^\bullet$ + 3-OHAA	7	410	0.1×10^8
$NO^\bullet$ + 3-OHAA	9	400	0.1×10^8

The formation and decay kinetics at 400 and 690 nm was found to be almost comparable (inset Fig. 3). Thus, it appears that the transient showing absorption maximum at 400 nm and wavelengths higher than 500 nm is similar. The bimolecular rate constants for the reaction with $N_3^\bullet$ radical is compiled in Table 1. Identical transient absorption spectrum was obtained on reaction of $^\bullet$OH radical with anthranilic acid (Supplementary Information. Fig. S1). Thus, it can be concluded that $^\bullet$OH radical leads to the complete oxidation of anthranilic acid as was observed in the case of aniline.[10–12,31] Due to the fact that $^\bullet$OH radical attacks at multi-sites which results in overlapping of transient absorption band,[10–12] the bimolecular rate constant was not determined.

The above observations can be summarized as: on comparing spectra of 3-OHAA and anthranilc acid obtained under identical conditions, confirm that the $^\bullet$OH radical on reaction with 3-OHAA leads to the formation of phenoxyl and cyclohexadienyl type of radical in addition to the formation of anilino radical while $N_3^\bullet$ radical on reaction with 3-OHAA leads to the formation of anilino radical. It is pertinent to mention here that in case of $N_3^\bullet$ radical reaction, presence of phenoxyl radicals especially on longer time scale cannot be excluded completely.

Reactions of $NO_2^\bullet$, $NO^\bullet$, and $CCl_3OO^\bullet$ radicals with 3-OHAA

The oxides of nitrogen are stable radicals and are a matter of concern due to vehicular and industrial pollution. In particular, $NO_2^\bullet$ is known to abstract H-atoms from unsaturated lipids, thus acting as an initiator of lipid per-oxidation. Besides, it adds to tyrosine to give 3-nitrotyrosine. 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.[33–35] One of the main causes of CCl_4-induced liver toxicity is metabolic activation of CCl_4 by cytochrome P-450 to initially form a free radical which then undergoes further reactions with lipids in presence of oxygen forming lipid peroxides.[36,37] Therefore, an attempt has been made to study the reactions of $CCl_3OO^\bullet$, $NO_2^\bullet$, and $NO^\bullet$ radicals with 3-OHAA.

The spectral and the kinetic data obtained on the oxidation of 3-OHAA by $CCl_3OO^\bullet$, $NO_2^\bullet$, and $NO^\bullet$ are summarized in Table 1. The rate constant values for the reaction of $CCl_3OO^\bullet$ and $NO_2^\bullet$ radical with 3-OHAA are of the order of $(1–6) \times 10^7\,dm^3\,mol^{-1}\,s^{-1}$. The $NO^\bullet$ radical seems to be innocuous and shows significant reaction with 3-OHAA only at pH 9.

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 and is strongly dependent on the electro-n-donating properties of the substituents and their position in the substrate. The reduction potential of 3-OHAA radical was determined by cyclic voltammetry (CV) of a deaerated aqueous solution containing 1×10^{-1} mol dm^{-3} KCl, 1.0×10^{-3} mol dm^{-3} phosphate buffer, and 1×10^{-3} mol dm^{-3} 3-OHAA at a scan rate of 50 mV/s. The differential pulse voltametric plot of 3-OHAA is shown in Fig. 4. Two clear peaks were observed at $E_1 = -0.30$ V and $E_2 = +0.29$ V. The reduction potential of 3-OHAA radical was determined from the maximum potential value (E_P), and was found to be $E = 0.53\,V \pm 0.06$ *versus* NHE. Cyclic voltammogram of this compound in similar solution has also been recorded from 0.8 V to −1.0 V (Supplementary Information, Fig. S2). Two reduction peaks in the forward and two oxidation peaks in the backward scan were observed. This indicates that the redox products are stable. In the backward scan, the first peak was not very prominent.

Repair reactions

The activated oxygen species formed as byproducts during the process of cellular respiration or as a result of interaction of ionizing radiation with biological molecules can damage the cell structures (*viz.*, proteins, DNA, lipids etc.). It is now well recognized that these processes ultimately lead to disease process and aging.[38–40] In the case of DNA, it is well established that the damage to the molecule by ionizing radiation is transmitted to the guanine moiety.[41] To study repair reactions, we have used guanosine ($E = 1.29$ V *vs.* NHE)[42,43] and chlorpromazine ($E = 0.86$ V *vs.* NHE)[44,45] as model compounds. Using pulse radiolysis after technique we have generated the free radicals of these compounds and then tried to assess their repair in the presence of 3-OHAA.

Repair reactions of chlorpromazine radical cation and guanosine radical

It was observed that in the presence of 3-OHAA (2×10^{-5}–1×10^{-4} mol dm^{-3}), the absorbance of guanosine

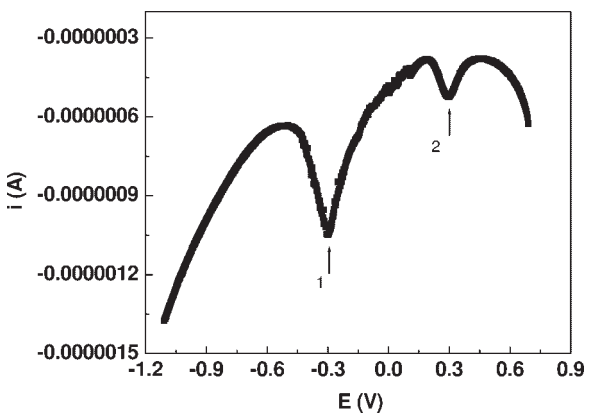

Figure 4. Differential pulse voltammogram of (1×10^{-3} mol dm^{-3}) 3-OHAA in N_2-bubbled aqueous solution containing (1×10^{-1} mol dm^{-3}) KCl and (1×10^{-3} mol dm^{-3}) phosphate buffer

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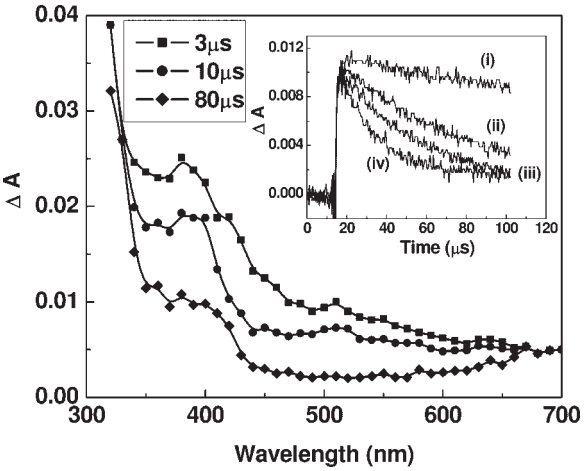

Figure 5. Transient absorption spectrum of guanosine radical generated by Tl^{2+} oxidation in a N_2O-saturated matrix containing (2×10^{-3} mol dm^{-3}) Tl^{+}, (5×10^{-4} mol dm^{-3}) guanosine, and (2×10^{-5} mol dm^{-3}) 3-OHAA at pH 7. (!) 3 μs (,) 10 μs (Λ) 80 μs after the 500 ns electron pulse. Inset: Decay traces of guanosine radical in the presence of various concentrations of 3-OHAA at 500 nm: (i) 0 mol dm^{-3}, (ii) 5×10^{-6} mol dm^{-3}, (iii) 1×10^{-5} mol dm^{-3}, and (iv) 2×10^{-5} mol dm^{-3}. Dose = 16 Gy/pulse

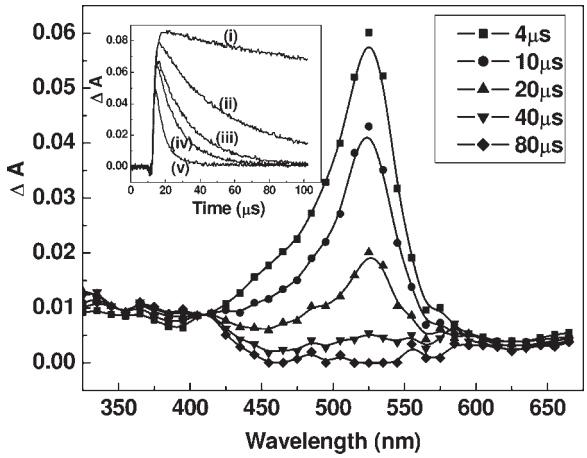

Figure 6. Transient absorption spectrum of $CPZ^{\bullet+}$ radical cation generated by $N_3^{\bullet}$ radical oxidation in a N_2O-saturated matrix containing (1×10^{-2} mol dm^{-3}) NaN_3, (1×10^{-4} mol dm^{-3}) CPZ, and (2×10^{-5} mol dm^{-3}) 3-OHAA at pH 5.5. Inset: Decay traces of $CPZ^{\bullet+}$ in the presence of various concentrations of 3-OHAA at 525 nm: (i) 0 mol dm^{-3}, (ii) 5×10^{-6} mol dm^{-3}, (iii) 1×10^{-5} mol dm^{-3}, (iv) 2×10^{-5} mol dm^{-3}, and (v) 5×10^{-5} mol dm^{-3}. Dose = 16 Gy/pulse

radical generated by the Tl^{2+} oxidation[46] of 5×10^{-4} mol dm^{-3} guanosine at 500 nm decayed exponentially (inset Fig. 5). It is pertinent to mention here that at pH 7, guanosine exists in the neutral form. Also, its radical generated after one electron oxidation has a pK_a of 3.9.[43] Therefore, at pH 7 the radical of guanosine[43] exists as $^{\bullet}$G (—H). Thus, it seems that on reaction of $^{\bullet}$G (—H) with 3-OHAA the following reaction takes place.

$$^{\bullet}G(-H) + 3-OHAA \rightarrow G + 3-OHAA^{\bullet} \qquad (11)$$

The bimolecular rate constant for the above reaction was calculated to be 2.4×10^9 dm^3 mol^{-1} s^{-1}. The transfer of H$^{\bullet}$ from 3-OHAA can take place at —NH_2 and —OH group. It is important to mention here that the involvement of proton transfer in the reductive repair of DNA guanyl radicals by aniline compounds has been suggested.[43] Based on this and our above observations, we feel that it is the —NH_2 and —OH groups which participate in the repair of guanosyl radical. To further substantiate this, a time resolved transient absorption spectrum was taken under identical conditions (Fig. 5). It can be seen that the absorbance yield at 500 nm becomes almost negligible while the absorbance yields at 400 nm and wavelength higher than 550 nm persists.

Phenothiazine drugs are generally used for psychiatric diseases and it has been suggested that free radical cations derived from them are important intermediates in their biochemical action.[44,45] Thus, we have attempted to explore the reactivity of chlorpromazine radical cation toward 3-OHAA. For this, initially chlorpromazine radical after one cation ($CPZ^{\bullet+}$) was generated under one electron oxidation conditions. It is known that at pH 7 chlorpromazine radical exists in cation form.[45] Thus, on one electron oxidation it generates a radical cation ($CPZ^{\bullet+}$) which shows absorption maximum at 270 and about 520 nm. It was observed that the absorbance yield at 525 nm decays exponentially with the increasing concentration of 3-OHAA. The results are shown in the inset of Fig. 6. The transient absorption spectrum of $CPZ^{\bullet+}$ radical cation in the presence of 3-OHAA is shown in Fig. 6. It can be seen that the absorbance yield due to radical cation becomes negligible by 80 μs. However, under identical time scale, the absorbance yield at *ca.* 400 nm increases. A clear isobestic point at 420 nm was observed indicating the transformation of one type of the transient to the other with concomitant decrease of the radical cation of $CPZ^{\bullet+}$. Since both phenoxyl and anilino radicals show absorption at wavelength *ca.* 400 nm and also, as the absorbance yield at wavelength higher than 575 nm by 80 μs do not become negligible, we presume that the repair of $CPZ^{\bullet+}$ radical cation is due to both the —NH_2 and —OH groups of 3-OHAA. The bimolecular rate constant for the repair reaction determined at 525 nm was found to be 4.4×10^9 dm^3 mol^{-1} s^{-1}.

SUMMARY

Although, the endogenously present tryptophan metabolite 3-OHAA is known to cause oxidation of proteins, the present pulse radiolysis study has shown its antioxidant defense mechanism by scavenging reactive oxygen and nitrogen species. Studies on one-electron oxidation reactions of 3-OHAA show that the oxidation takes place at both the –NH_2 and –OH groups. Another point of interest is its role in carrying out repair reaction of model target bio-molecules at physiological pH. We have initiated theoretical study on related compounds to explore more on the observed phenomenon.

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