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Redox chemistry of 8-azaadenine: a pulse radiolysis study

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ABSTRACT: Aza derivatives of purines and pyrimidines are important class of compounds, which are known for their cytotoxic, antimicrobial, and mutagenic activities. The redox chemistry of 8-azaadenine (8AA) has been investigated using pulse radiolysis technique. The oxidation reactions were studied using hydroxyl radical ($^{\bullet}OH$), oxide radical anion ($O^{\bullet -}$ and sulfate radical anion ($SO_4^{\bullet -}$), and the reduction reactions were studied using hydrated electron (e_{aq}^-) and hydrogen radical ($H^{\bullet}$). In the reaction of $^{\bullet}OH$, a bimolecular rate constant of $3.8 \times 10^8\ dm^3\ mol^{-1}\ s^{-1}$ was determined at pH 6.0. The transient spectrum obtained for the reaction of $^{\bullet}OH$ at pH 6 has an absorption maximum around 340 nm and is assigned to the formation of 8AA-4OH$^{\bullet}$. The charge population density was calculated theoretically (using Gaussian 98) and it showed that the fourth carbon atom (C(4)) is the most probable site for the attack of $^{\bullet}OH$. The oxidizing nature of this radical is demonstrated by its reaction with *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD). The existence of this species is further supported by theoretical calculations where the absorption maximum of this radical is calculated as 338 nm. The yield of 8AA-4OH$^{\bullet}$ is estimated as around 85%. At pH 10.2, the transient spectrum with maxima at 300 and 350 nm is attributed to the dehydrated 8AA-4OH$^{\bullet}$, which is an N-centered radical of the type 8AA-N(9)$^{\bullet}$. In the reaction of $O^{\bullet -}$ (pH $\approx$ 14) a transient spectrum with similar features is observed. Therefore this is also assigned to 8AA-N(9)$^{\bullet}$. A bimolecular rate constant for this reaction is determined as $4.2 \times 10^8\ dm^3\ mol^{-1}\ s^{-1}$. In the reaction of $SO_4^{\bullet -}$ at pH 6, the transient spectrum having λ_{max} at 320 nm is attributed to the formation of a neutral radical of 8AA (8AA-N(6)$^{\bullet}$), which is formed by the deprotonation of the initially formed radical cation. But at pH 10.2, the spectrum is found to be similar to the one observed in the reaction of $O^{\bullet -}$ and hence it is assigned to the formation of the nitrogen-centered radical 8AA-N(9)$^{\bullet}$. In the reaction of e_{aq}^-, a second-order rate constant of $1.8 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$ is determined at pH 6 and the transient absorption spectrum with λ_{max} at 330 nm is assigned to the protonated electron adduct of 8AA (8AA(NH$^{\bullet}$)). The reducing nature of this intermediate is confirmed by the formation of methyl viologen radical cation ($MV^{\bullet +}$) from its reaction with MV^{2+}. The transient intermediate in the case of the reaction of $H^{\bullet}$ is proposed as 8AA-C2(H)N(3)$^{\bullet}$ at pH 1. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: 8-Azaadenine; redox chemistry; free radicals; radiation chemical; pulse radiolysis; OH-adducts; N-centered radicals; C-centered radicals

INTRODUCTION

Development of chemical compounds, which have similar structures of nucleic acid components such as purines and pyrimidines for testing their antimetabolic activity, is important to obstruct the abnormal development of cancer cells. Among these compounds, aza derivatives have shown promising results in the clinical investigations because of their cytotoxic, antimicrobial, and mutagenic activities.[1,2] Free radical chemistry of the aza compounds is perceptibly of great importance as tumor treatment involves the simultaneous use of both chemo and radiotherapy. Despite the importance in cancer therapy,[3] their free radical chemistry remains poorly understood. An earlier study[4] reported that the water derived free radicals such as hydroxyl radicals ($^{\bullet}OH$) and hydrated electrons (e_{aq}^-) react with aza analogous of pyrimidine at diffusion controlled rate (10^9–$10^{10}\ dm^3\ mol^{-1}\ s^{-1}$) at pH 8. This second-order rate constant is well comparable to the rates with nucleobases such as purines and pyrimidines.

The general radical chemistry of adenine is well documented.[5,6] For example, the potential sites of attack in the case of the reaction of $^{\bullet}OH$ with adenine are identified as C4, C5, and C8 positions (Scheme 1).[7–9] $^{\bullet}OH$

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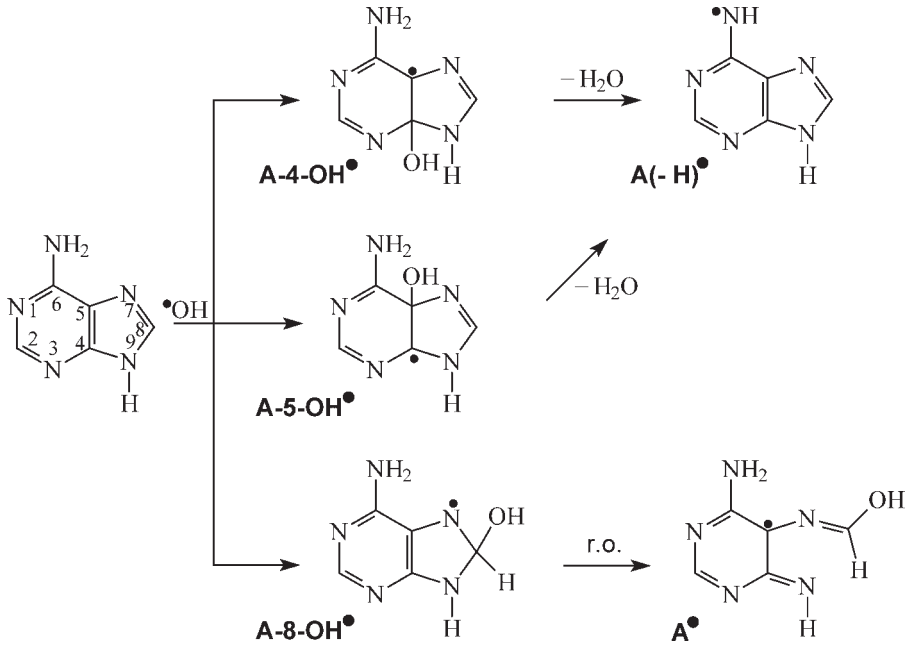


Scheme 1. Mechanism of the reaction of •OH with adenine[7,8] (r.o. represents ring opening reaction)

adds to C(4) (35% probability), to C(5) (19%) and to C(8) (30%) in the case of N^6,N^6-dimethyladenosine.[8] The resulting radicals are represented as A-4OH•, A-5OH• and A-8OH•, and all of these undergo unimolecular transformation reactions. The A-4OH• undergoes a fast dehydration reaction leading to the formation of a nitrogen-centered radical (Scheme 1).[7,8] The A-8OH• undergoes a ring opening reaction (imidazole ring), which yields 5-formamido-6-aminopyrimidine type product (FAPy). In addition to this, the A-8OH• leads to an important product called 8-hydroxy adenine (analogous reaction in guanine leads to the generally known 8-oxoguanine which is promutagenic in nature).[6] These two products are expected to be absent with 8-azaadenine (8AA) as the 8th carbon position is replaced by a nitrogen. In the case of caffeine (a substituted purine and biologically important molecule) only C(4) and C(8) OH-adducts are formed with a ratio of 1:2.[10] While the formation of A-8OH• is an important reaction in the case of adenine and other purine systems, such a reaction could be completely blocked by the presence of nitrogen at the 8th position of 8AA. This would result an entirely different radical chemistry of 8AA compared to adenine.

Similarly the reaction of other free radicals such as oxide radical anion ($O^{\bullet -}$), sulfate radical anion ($SO_4^{\bullet -}$), hydrated electron (e^-_{aq}), and hydrogen atom ($H^\bullet$), could be interestingly different from adenine derivatives. Therefore, a detailed radiation chemical study of 8AA is carried out using both oxidizing and reducing radicals and identified the various intermediates formed from the reaction of these free radicals in aqueous medium using the technique of pulse radiolysis. Some of the experimental results have been supported by theoretical calculations.

EXPERIMENTAL

8-Azaadenine (8AA), *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD), methyl viologen (MV^{2+}) and potassium peroxodisulphate ($K_2S_2O_8$) were purchased from Aldrich and were used without further purification. All solutions were prepared in water purified by Millipore Milli-Q system. The concentration of the substrate was kept at 1×10^{-3} mol dm^{-3}. Radiolysis of water produces highly reactive radicals e^-_{aq}, $H^\bullet$, and •OH in addition to the formation of inert or less reactive molecular products such as H_2 and H_2O_2.[6]

$$H_2O \xrightarrow{\sim\sim\sim} e^-_{aq}, H^\bullet, {}^\bullet OH, H_2, H_2O_2, H_3O^+ \quad (1)$$

The reaction of •OH was carried out in N_2O saturated solutions where e^-_{aq} is quantitatively converted to •OH (reaction 2).[6] The reaction of $O^{\bullet -}$ radicals was investigated in N_2O saturated aqueous solution at pH ≈ 14 since •OH is in equilibrium with its basic form $O^{\bullet -}$ at highly basic medium (reaction 3, pK_a = 11.9). $SO_4^{\bullet -}$, radicals were produced by the radiolysis of N_2 saturated aqueous solution containing 2-methyl-2-propanol (0.2 mol dm^{-3}) and $S_2O_8^{2-}$ (5×10^{-2} mol dm^{-3}) (reaction 4 and 5). The reaction of e^-_{aq} was studied in N_2 saturated aqueous solution containing 2-methyl-2-propanol which acts as a •OH scavenger. The reaction of

$H^{\bullet}$ was investigated at pH 1 in N_2O saturated solutions in the presence of 2-methyl-2-propanol as $^{\bullet}OH$ scavenger.

$$N_2O + e^-_{aq} \rightarrow {}^{\bullet}OH + OH^- + N_2 \quad (2)$$

$$^{\bullet}OH + OH^- \rightleftharpoons O^{\bullet -} + H_2O \quad (3)$$

$$e^-_{aq} + S_2O_8^{2-} \rightarrow SO_4^{\bullet -} + SO_4^{2-} \quad (4)$$

$$^{\bullet}H + S_2O_8^{2-} \rightarrow SO_4^{\bullet -} + SO_4^{2-} + H^+ \quad (5)$$

Pulse radiolysis experiments were carried out using a linear accelerator delivering electron pulses of 7 MeV energy of 50 nsec duration. An aerated aqueous solution of KSCN (1×10^{-2} mol dm^{-3}) was used to monitor the dose per pulse with $G \times \varepsilon_{500} = 21\,520\ dm^3\ mol^{-1}\ cm^{-1}$ and was normally kept at 10–12 Gy. A low dose per pulse of 6.5 Gy was used for the investigation of the electron transfer reaction between the electron adducts and MV^{2+} and between OH adducts and TMPD. The transient species formed on pulse radiolysis were monitored by using a 450 W pulsed xenon lamp, a monochromator (Kratos GM-252) and a Hamamatsu R-955 photomultiplier as the detector. The photomultiplier output was digitized with a 100 MHz storage oscilloscope interfaced to a computer for kinetic analysis. The details of the pulse radiolysis set-up have been described elsewhere.[11]

Theoretical calculations

Theoretical calculations were performed with the B3LYP hybrid density functional theory.[12] All the geometries were optimized using the B3LYP/6-31+G* (5d) level. NBO charge densities and spin populations were calculated at the same level. NBO calculations were carried out using NBO 4.0 interfaced to Gaussian 98.[13] The electronic spectra were computed using Random Phase Approximation methods applied at B3LYP/6-31+G* level of theory.

RESULTS AND DISCUSSION

Reactions of $^{\bullet}OH$

The transient absorption spectrum recorded at 5 μsec after the pulse is characterized by its maximum at 340 nm and a broad absorption centered around 580 nm (pH 6). The rate of initial absorption build-up of the transients (k_{obs}) was found to be linearly dependent on the concentration of 8AA and from this dependence at 340 nm, a bimolecular rate constant of $3.8 \times 10^8\ dm^3\ mol^{-1}\ s^{-1}$ was determined at pH 6.0. This rate constant is lower compared to adenine ($6 \times 10^9\ dm^3\ mol^{-1}\ s^{-1}$)[14] and is well comparable to purine ($3 \times 10^8\ dm^3\ mol^{-1}\ s^{-1}$).[14] The lower rate constant for the electrophilic attack of $^{\bullet}OH$ compared to adenine is understandable because of the presence of a nitrogen at the 8th position instead of carbon which makes the compound more electron deficient compared to adenine. In addition to this, the three probable sites of addition of $^{\bullet}OH$ in adenine (C4, C5, and C6 positions) has been reduced to two (C4 and C5) which may also affect the rate of reaction. The details of the sites of addition are discussed in the following section.

The absorption spectrum obtained at pH 6 at 40 μsec after the pulse has a well-defined maximum around 340 nm (Fig. 2). The time resolved spectra however did not show any absorption changes up to several hundreds of microseconds other than a second-order decay. This spectral behavior indicates a clear distinction from the behavior of the transients from adenine, where the initial spectrum undergoes a transformation at higher time scales.[7–9] To look at the reactivity of the intermediate with oxygen, the spectra were also recorded in the presence of oxygen, however no significant change in the spectral properties was observed (data not shown). The reactivity of oxygen is a good indication of the reducing property of the intermediate and it is known to have high reactivity with C-centered organic radicals ($k \sim 10^9\ dm^3\ mol^{-1}\ s^{-1}$).[15] The spectral features at higher pH (pH 10.2) were very different compared to that at pH 6. The transient spectrum recorded at 40 μsec after the pulse showed two distinct absorption maxima at 300 and 350 nm and a broad maximum centered around 640 nm (Fig. 2). This spectrum undergoes a second-order decay at higher time scales.

One of the easiest methods to understand the redox nature of the transient intermediates is the use of known oxidants/reductants at sufficiently low concentrations so that there will not be any direct reaction of $^{\bullet}OH$ with the oxidants/reductants, while the intermediates would react with the oxidants/reductants making use of their slight differences in the oxidation/reduction potentials.[6] Therefore, in the present case, *N*,*N*,*N*′,*N*′-tetramethyl *p*-phenylenediamine (TMPD) was used as a reductant to explore the oxidizing nature of the intermediate radicals. A strong absorption build-up of the radical cation of TMPD ($TMPD^{\bullet +}$) which is typical of similar electron transfer reaction from the oxidizing intermediate to TMPD,[7–9] has been observed at 565 nm at pH 6 (Fig. 2, inset). The yield of $TMPD^{\bullet +}$ was also calculated in terms of $G(TMPD^{\bullet +})$ and was 4.5×10^{-7} mol J^{-1}. The total yield of $^{\bullet}OH$ ($G(^{\bullet}OH)$) is calculated as 5.3×10^{-7} mol J^{-1} which is based on the concentration of 8AA and its second-order rate constant.[16] Considering this G value, the observed $G(TMPD^{\bullet +})$ constitutes about 85% of the total reaction. A similarly high $G(TMPD^{\bullet +})$ of 5.0×10^{-7} mol J^{-1} was obtained at pH 10.2 as well, which constitutes about 94% of the total $^{\bullet}OH$ reaction.

The charge population density, calculated for 8AA is shown in Fig. 1. As can be seen from the figure that the calculated NBO charge population at C(4), C(5), and N(8) are 0.35, −0.01, and −0.02 au, respectively. These sites are specifically highlighted (Fig. 1) because of the fact that the

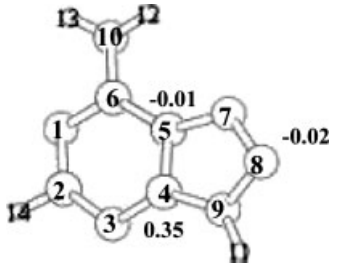


Figure 1. NBO Charge population (in au) of 8-azaadenine (this demonstrates the C(4) (0.35 au) is the most probable site of electrophillic attack)

potential sites of $^{\bullet}$OH attack in adenine derivatives are C(4), C(5), and C(8).[7–9] As the reaction of $^{\bullet}$OH is an electrophilic addition, it is clear that C(4) is the most probable site for the attack of $^{\bullet}$OH. This is an additional support for the conclusion that the major OH-adduct from the reaction of $^{\bullet}$OH is C(4)-OHC(5)-yl radical.

One of the general phenomena observed with adenine, adenosine, and their substituted derivatives is a unimolecular transformation of the OH adduct at neutral pH.[7–9] Such a phenomenon is completely absent in 8AA as was observed from the time resolved spectra. The transient spectrum, shown in Fig. 2, therefore, represents a relatively stable species in the time scale of pulse radiolysis. From the electron density calculations as well as from the earlier reports on the purine derivatives,[7–9] it is proposed that the main OH-adduct, produced immediately after the pulse, is the C(4)-OHC(5)-yl radical (8AA-4OH$^{\bullet}$). The assignment of this radical structure can be supported from its reaction with TMPD where the clear electron transfer from TMPD (Fig. 2) demonstrates its oxidizing nature. This oxidizing property of the radical can well be explained because of the presence of unpaired spin density on N1, N3 or N8 in all the other possible mesomeric structures (Scheme 2). The existence of 8AA-4OH$^{\bullet}$ is further supported by theoretical calculations where the absorption maximum of this radical is calculated as 338 nm. This value matches well with the observed absorption maximum at 340 nm (Fig. 2). Finally, an additional support to the existence of 8AA-4OH$^{\bullet}$ is its stability in the presence of O_2. The A-4OH$^{\bullet}$ from adenine derivatives was shown to be stable in the presence of O_2.[7–9] Being an oxidizing radical with mesomeric structures having unpaired spin density at nitrogen, it is likely to possess a low reactivity towards oxygen. The nitrogen-centered radicals are reported to have rate constant less than $10^7\,dm^3\,mol^{-1}\,s^{-1}$.[16] Though, a water elimination from this species is quite possible, the experimental evidences take us to a conclusion that such a phenomenon takes place only at a later stage (>200 μsec) and that the 8AA-4OH$^{\bullet}$ is stable compared to its adenine counterpart. On the other hand, the possibility of the formation of 8AA-5OH$^{\bullet}$ cannot be ruled out. However 8AA-5OH$^{\bullet}$ cannot be an oxidant unlike 8AA-4OH$^{\bullet}$. The yield of TMPD$^{\bullet+}$ at pH 6 ($G(TMPD^{\bullet+}) = 4.5 \times 10^{-7}\,mol\,J^{-1}$) is about 15% less than the quantitative yield of $^{\bullet}$OH. It is therefore reasonable to assume that this reduction in the quantitative yield of TMPD$^{\bullet+}$ resulted from the formation of 8AA-5OH$^{\bullet}$. However, it is unlikely that this can contribute significantly to the absorption spectrum because of its low yield. Moreover, the theoretical calculations showed that 8AA-5OH$^{\bullet}$ has an absorption maximum at 318 nm. However, no such indication is obtained from the observed spectrum (Fig. 2). An absorption coefficient of 8AA-4OH$^{\bullet}$ is approximately calculated as $1640\,dm^3\,mol^{-1}\,cm^{-1}$ at 340 nm.

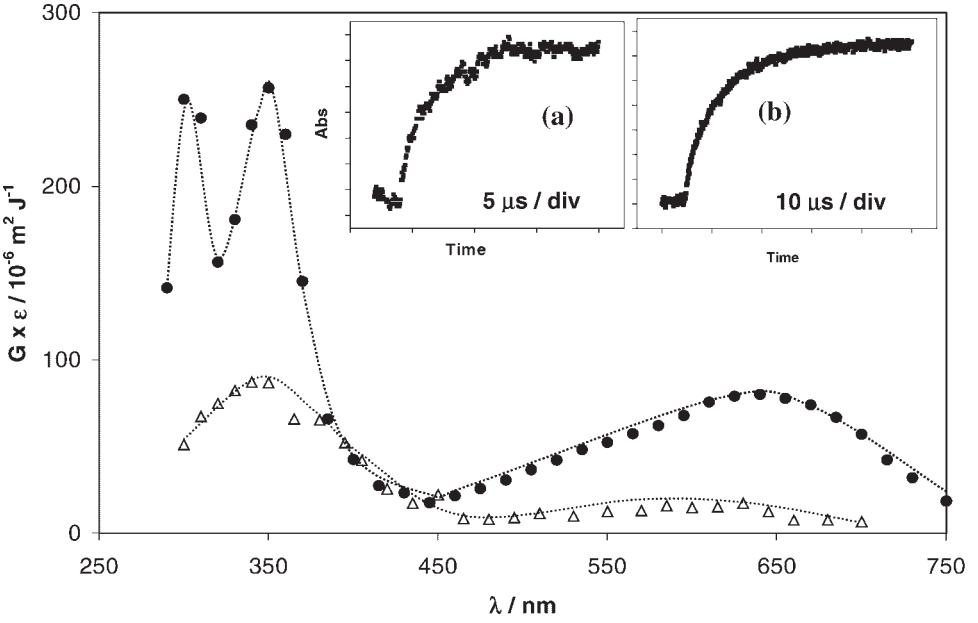
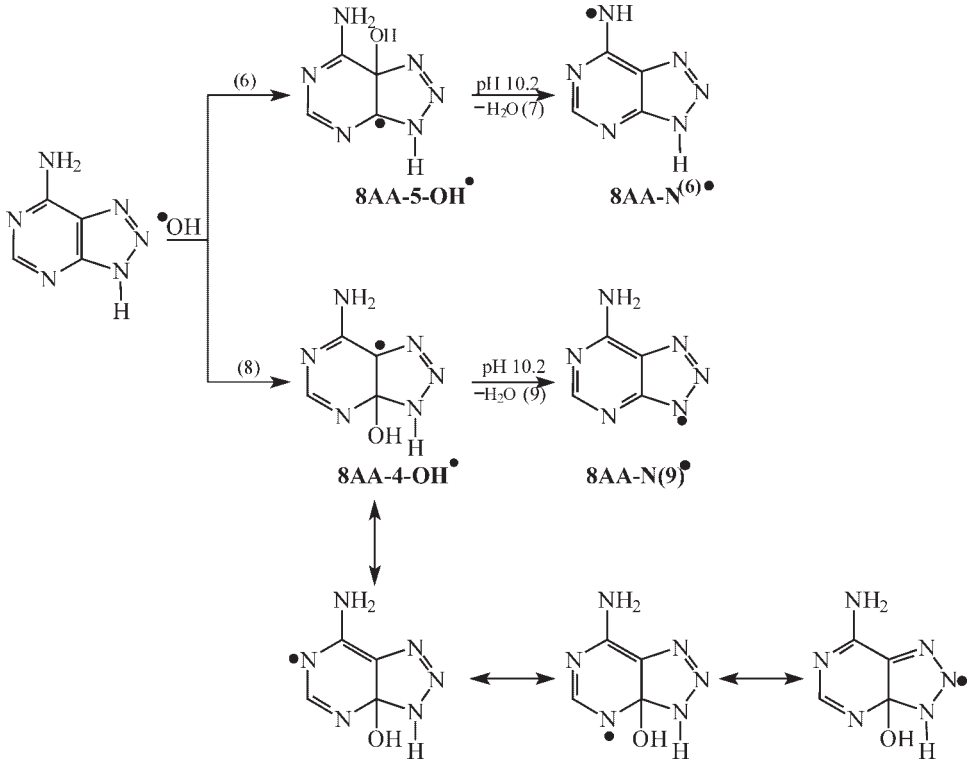


Figure 2. Reaction of hydroxyl radical ($^{\bullet}$OH): Transient absorption spectra recorded in N_2O saturated aqueous solutions of 8 azaadenine ($1 \times 10^{-3}\,mol\,dm^{-3}$) at 40 μsec after the pulse at pH 6(△) and pH 10.2 (●). Inset: (a) Formation trace at 340 nm at pH 6, (b) absorption build-up of TMPD$^{\bullet+}$ from the reaction between the oxidizing intermediate and TMPD at 565 nm at pH 6



Scheme 2. Proposed mechanism of the reaction of $^{\bullet}OH$ with 8-azaadenine

The expected dehydration from 8AA-4OH$^{\bullet}$ is likely to get enhanced at basic pH and the spectral behavior at pH 10.2 is in line with this fact. The nearly quantitative yield of $TMPD^{\bullet+}$ ($G(TMPD^{\bullet+}) = 5 \times 10^{-7}\,mol\,J^{-1}$) demonstrates the oxidizing property of the intermediate. The spectral behavior at pH 10.2 is very different compared to that at pH 6 (Fig. 2). The absorption spectrum with maxima at 300, 350, and 640 nm is attributed to the dehydrated 8AA-4OH$^{\bullet}$ which is an N-centered radical and can be represented as 8AA-N(9)$^{\bullet}$ (Scheme 1). This result is, thus, very different from the reaction of $^{\bullet}OH$ with adenine and its derivatives[7–9]at higher pH where an OH^- induced inhibition of OH^- elimination of A-4OH$^{\bullet}$ leading to a deprotonated radical $A\text{-}4O^{\bullet-}$ is reported. Because of this reason the yield of $TMPD^{\bullet+}$ is seen highly reduced at higher pH.[7–9] The existence of 8AA-N(9)$^{\bullet}$ in the present case is further confirmed by the formation of a transient species from the reaction $O^{\bullet-}$ with 8AA which has very similar spectral characteristics (see Reaction of Oxide Radical Anion ($O^{\bullet-}$)). The reaction of $O^{\bullet-}$ with nucleobases proceeds via an electron transfer reaction with the deprotonated 8AA at pH ≈ 14 (8AA has a pK_a at 11.5) leading to a N(9)-centered radical.[18] The OH^- induced dehydration reaction from 8AA-5OH$^{\bullet}$, though formed low in yield, is equally likely. The so formed 8AA-N(6)$^{\bullet}$ (Scheme 2) can be a very good oxidant similar to 8AA-N(9)$^{\bullet}$ which explains the high yield of $TMPD^{\bullet+}$ at higher pH. As shown in Scheme 2, the dehydration reaction can lead to two possible structures such as 8AA-N(6)$^{\bullet}$ and 8AA-N(9)$^{\bullet}$. However, it is difficult to clearly distinguish the contribution of these two radicals as both can be equally reactive to TMPD. Since the yield of 8AA-5OH$^{\bullet}$ is expected to be less than 15% (see previous paragraph) it can be concluded that the spectral contribution is largely from 8AA-N(9)$^{\bullet}$. Furthermore, the calculated absorption maxima of this radical are 325, 335, and 628 nm. Although the first maximum at 300 nm is not near to the calculated value, the rest of the two significantly match with the calculated values.

Reaction of oxide radical anion ($O^{\bullet-}$)

Oxide radical anion is nucleophilic in nature unlike the electrophilic hydroxyl radical. The reactions of $O^{\bullet-}$, which is a conjugate base of $^{\bullet}OH$, will be quite different from that initiated by $^{\bullet}OH$. It is an oxidant ($E^0(O^{\bullet-},H^+/OH^-) = 1.77$ V).[17] Its reactions are normally carried out at a pH greater than 12 as the pK_a value of $^{\bullet}OH \rightleftharpoons O^{\bullet-} + H^+$ is at 11.9.[6] Therefore, the reaction of $O^{\bullet-}$ with 8AA is carried out at pH ≈ 14.[18] A second-order rate constant of $4.2 \times 10^8\,dm^3\,mol^{-1}\,s^{-1}$ is determined from the pseudo first-order absorption build-up of the intermediate with respect to the concentration of 8AA at 350 nm. The transient absorption spectrum at 40 μsec after the pulse has shown similar features like the spectrum obtained from the reaction of $^{\bullet}OH$ at pH 10.2, with absorption maxima at 300 and 350 nm with a broad absorption centered around 640 nm (Fig. 3). The absorption traces obtained at these wavelengths showed only a second-order decay. This observation was however different compared to the case with adenine where the

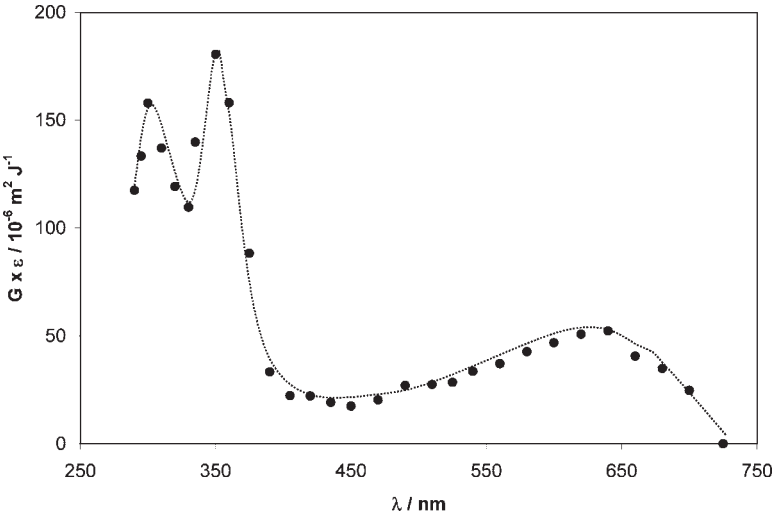
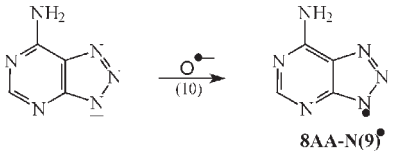


Figure 3. Reaction of oxide radical anion ($O^{\bullet -}$): Transient absorption spectrum recorded in N_2O saturated aqueous solutions of 8 azaadenine (1×10^{-3} mol dm^{-3}) at 40 μsec after the pulse at pH ≈ 14

OH-adduct undergoes a first-order type decay and form a hydrated adduct.[19] The electron transfer reactions from the intermediates to TMPD was also carried out and a strong absorption build-up of $TMPD^{\bullet +}$, very similar to the reaction of $^{\bullet}OH$ at pH 10.2, was obtained.

At pH ≈ 14, 8AA is predominantly existing in its deprotonated form at nitrogen as it has a pK_a at 11.5. The reaction of $O^{\bullet -}$ was reported to be very different from the reaction of $^{\bullet}OH$ as the former undergoes either electron transfer or a hydrogen abstraction reaction with adenine and other nucleobases.[19–21] It is therefore proposed that $O^{\bullet -}$ undergoes an electron transfer reaction at N9 and this results the formation of a nitrogen-centered radical, 8AA-N(9)$^{\bullet}$ (reaction 10). The deprotonated adenine (at N(9)) has similar reaction with $O^{\bullet -}$ at pH > 13.[18] In the case of $O^{\bullet -}$ a similar $TMPD^{\bullet +}$ build up is observed as in the reaction of $^{\bullet}OH$ (data not shown). The formation of $TMPD^{\bullet +}$ can be well explained based on the oxidative nature of 8AA-N(9)$^{\bullet}$ and this is an additional support for the interpretation. Furthermore, it can be easily understood that this is the same radical species, which is formed after the dehydration reaction of the 8AA-4OH$^{\bullet}$ at pH 10.2 (Scheme 2). An absorption coefficient of 2670 dm^3 mol^{-1} cm^{-1} is calculated for the 8AA-N(9)$^{\bullet}$ at 300 nm.



Reaction of sulfate radical anion ($SO_4^{\bullet -}$)

The $SO_4^{\bullet -}$ is a powerful oxidizing radical with an oxidation potential of 2.5–3.1 V/NHE.[22] This radical is frequently used in the study of DNA damage as it can produce a DNA radical cation and an electron in aqueous medium,[6] which is a similar process like the *direct effect* of ionizing radiation. In the present study, the reaction of $SO_4^{\bullet -}$ is carried out at near neutral as well as at basic pH. A second-order rate constant of 2.1×10^8 dm^3 mol^{-1} s^{-1} is determined at pH 6 from the rate of build-up of the transient intermediate at 320 nm. Similar to the rate of reaction of $^{\bullet}OH$, the rate constant value is lower by an order of magnitude compared to adenine ($k = 4.6 \times 10^9$ dm^3 mol^{-1} s^{-1})[14] while almost of the same order compared to purine ($k = 3.0 \times 10^8$ dm^3 mol^{-1} s^{-1}). This is due to the reduction in the electron donating nature because of the presence of an additional nitrogen compared to adenine. A comparatively weak transient absorption spectrum is obtained at pH 6 with a single maximum at 320 nm (Fig. 4). However, when the pH is raised to 10.2, the spectral features were very different with two prominent absorption maxima at 300 and 350 nm and a (weak) broad maximum centered around 650 nm (Fig. 4). It is, however, noticeable that this spectrum matches well with the one obtained from the reaction of $^{\bullet}OH$ at pH 10.2 and of $O^{\bullet -}$ at pH ≈ 14 (Figs. 2 and 3).

Being a very powerful oxidant, $SO_4^{\bullet -}$ undergoes one-electron oxidation with purines either by outer-sphere electron transfer or by inner-sphere electron transfer mechanism.[9,23] The primary product of oxidation by $SO_4^{\bullet -}$ is a radical cation, which possess a very short life time in aqueous solutions in the case of purine systems because of their high Bronsted acidity.[5] Therefore, they deprotonate very fast ($k > 10^7$ sec), resulting a neutral radical.[7] Furthermore, the pK_a of the radical cation is reported as much lower than 1.[5] In line with these information, in the present case, a radical cation of 8AA is expected to be formed in the first place which may undergo, in principle, a number of reactions in aqueous state such as deprotonation to form a neutral radical and

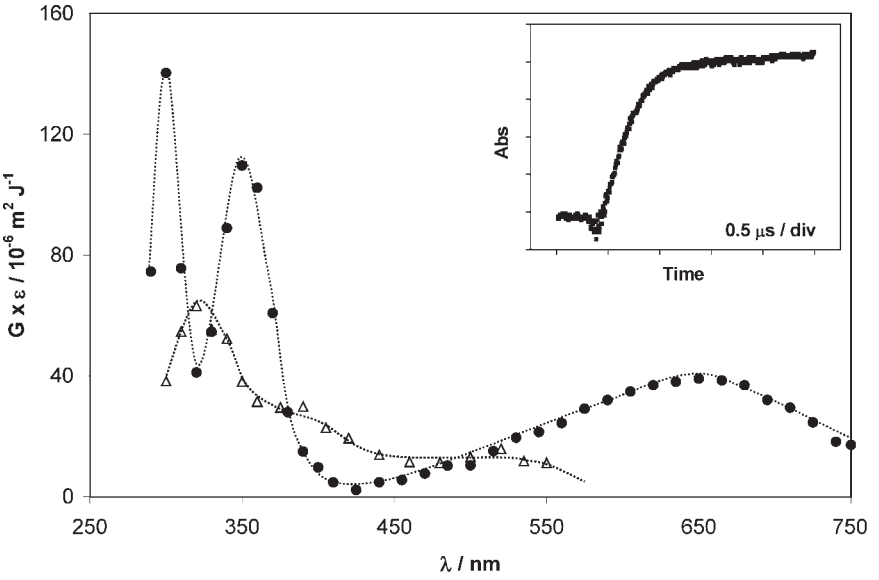
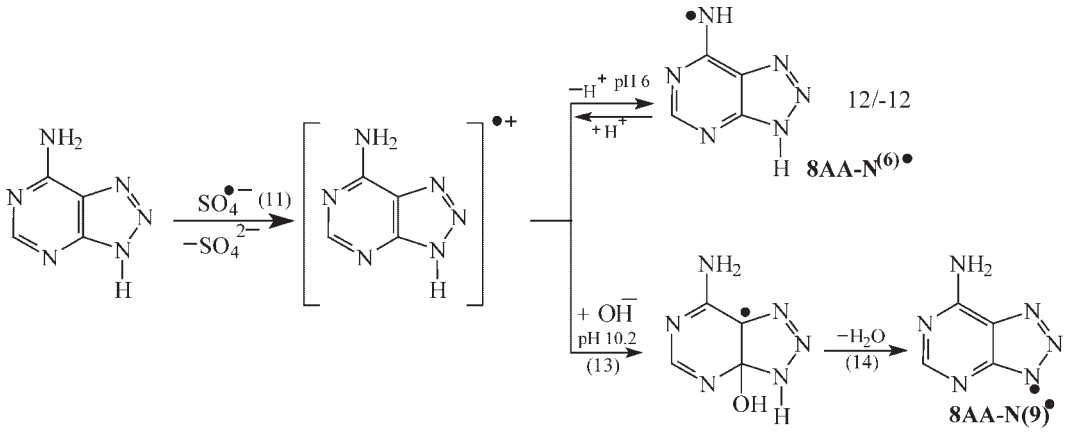


Figure 4. Reaction of sulfate radical anion ($SO_4^{\bullet -}$): Transient absorption spectra recorder in N_2 saturated aqueous solutions of 8 azaadenine (1×10^{-3} mol dm^{-3}) containing 2-methyl-2-propanol (0.2 mol dm^{-3}) and $S_2O_8^{2-}$ (1×10^{-2} mol dm^{-3}) at 40 μsec after the pulse at pH 6 (△) and at pH 10.2 (●). Inset: absorption build-up of the intermediate at 300 nm at pH 10.2

reaction with water or OH^- at basic pH to form the corresponding OH-adducts. If latter is the case, the spectral features at pH 6 should match with the reaction of $^{\bullet}OH$ at the same pH. However, this is not the case as can be seen from Figs. 2 and 4. Then the most likely mechanism is the deprotonation of the radical cation to form a neutral radical of the form 8AA-N(6)$^{\bullet}$ (Scheme 3). The deprotonation of the radical cation of adenine derivatives at the N^6-position[5] supports such an assignment of the intermediate spectrum. However, the situation is quite different at pH 10.2, where the spectral features favor a mechanism where the radical cation reacts with the OH^- leading to the OH-adduct. The transient spectra from the reaction of $^{\bullet}OH$ (at pH 10.2), $O^{\bullet -}$, and $SO_4^{\bullet -}$ (at pH 10.2) are well comparable as can be seen from Figs. 2, 3 and 4. It is therefore proposed that the initially formed radical cation reacts with OH^- and forms 8AA-4OH$^{\bullet}$ which on dehydration reaction to give rise to a nitrogen-centered radical of the form 8AA-N(9)$^{\bullet}$ as shown in Scheme 3. In addition to 8AA-4OH$^{\bullet}$ it is probable that 8AA-5OH$^{\bullet}$ could also be formed from the addition of OH^- at the C5 position. However, the spectral similarity at this pH with that from the reaction of $^{\bullet}OH$ at pH 10.2 and of $O^{\bullet -}$, is clearly in line with the interpretation of the formation of 8AA-4OH$^{\bullet}$ as the precursor of 8AA-N(9)$^{\bullet}$. The spectral contribution from 8AA-5OH$^{\bullet}$ is thus proposed as insignificant.

Reactions of hydrated electrons (e_{aq}^-) and hydrogen atom ($^{\bullet}H$)

In general, the purines have high intrinsic reaction with e_{aq}^- ($k \approx 10^{10}$ dm^3 mol^{-1} s^{-1}) because of the presence of



Scheme 3. Proposed mechanism of the reaction of $SO_4^{\bullet -}$ with 8-azaadenine

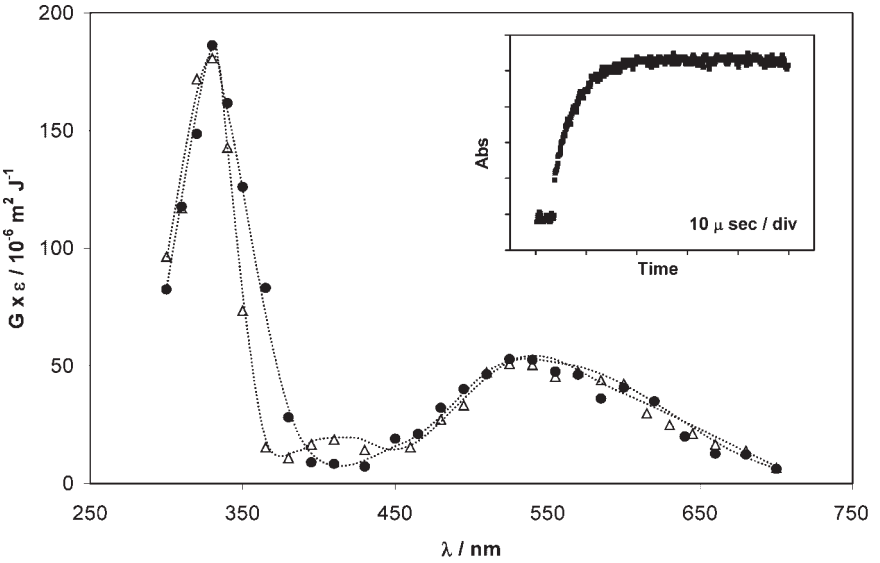
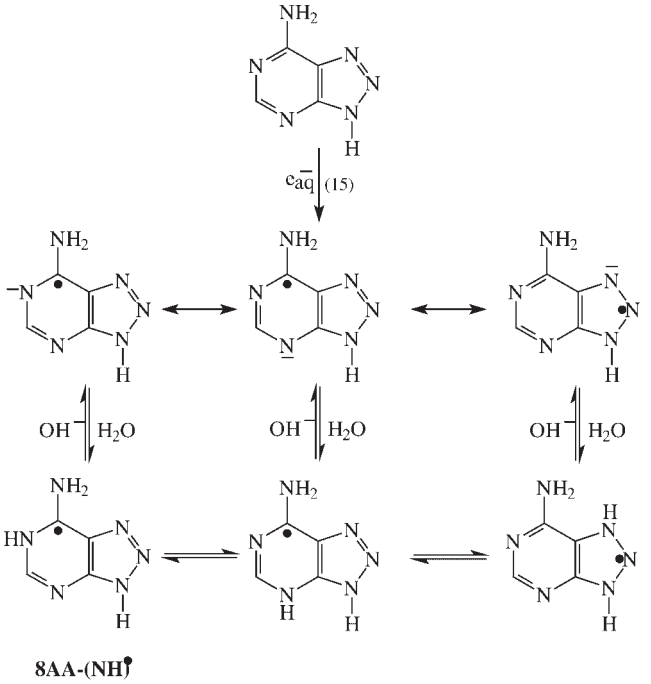


Figure 5. Reaction of hydrated electron (e_{aq}^-): Transient absorption spectra recorded in N_2 saturated aqueous solutions of 8 azaadenine (1×10^{-3} mol dm^{-3}) containing 2-methyl-2-propanol (0.2 mol dm^{-3}) at 3 μsec after the pulse at pH 6 (△) and pH 12 (●). Inset: Absorption build-up of $MV^{\bullet+}$ from the reaction of the reducing intermediate and MV^{2+} at 605 nm at pH 6

electron deficient pyrimidine ring.[14] 8AA has also shown similar reactivity. A second-order rate constant of 1.8×10^{10} dm^3 mol^{-1} s^{-1} is determined at pH 6 from the pseudo first-order decay of e_{aq}^- with respect to the concentration of 8AA at 720 nm. The transient absorption spectrum showed a major absorption maximum at 330 nm and a broad absorption in the 500–600 nm region (Fig. 5). A similar spectrum is obtained when the pH is raised to 12 where 8AA exists in its deprotonated form. In both the pH, the spectra underwent a second-order decay. The reducing nature of the intermediate is confirmed by the formation of methyl viologen radical cation ($MV^{\bullet+}$) at 605 nm at pH 6 (Fig. 5), resulting from the reaction of an electron transfer from the reducing intermediate to the oxidant, MV^{2+}. A G($MV^{\bullet+}$) (at pH 6 as well as at 12) of 2.4×10^{-7} mol J^{-1} is obtained which constitute about 89% of the total reaction. A weak transient absorption spectrum is obtained for the reaction of H$^\bullet$ with 8AA at acidic pH (pH 1) with a single maximum at around 330 nm (data not shown).

The spectral features observed in this case are similar to the adenine, hypoxanthine, and their derivatives. The electron adducts of these compounds, being much stronger bases compared to the pyrimidine bases, rapidly undergoes protonation by water ($k \geq 10^7$ sec^{-1}).[24–27] A similar situation is expected with 8AA as well. The initially formed electron adduct may rapidly get protonated by water at pH 6. Therefore, the transient spectrum with λ_{max} at 330 nm is assigned to the protonated electron adduct of 8AA. As the potential site of electron attack is at nitrogen, a nitrogen protonated carbon-centered radical is proposed (8AA(NH$^\bullet$)) (Scheme 4). A strong evidence for the spin density at carbon came from the quantitative electron transfer from this radical to MV^{2+} (Fig. 5) as carbon-centered radical can act as an electron donor. Such properties were unambiguously demonstrated using the oxidants MV^{2+}, *p*NAP (*p*-nitroacetophenone), etc. in the case of adenine and its derivatives.[5,24,25] However, there are three similar sites of electron attack and hence the electron adducts must be represented by their mesomeric structures as shown in the Scheme 4. Correspondingly the protonated counterparts would exist in their tautomeric forms (Scheme 4). Unlike the protonation at nitrogen, the protonation at carbon is much slower, though carbon-protonated species having higher pK_a would be thermodynamically more favored. Such carbon protonation reactions catalyzed by OH^- and $HPO_4^{2-}/H_2PO_4^-$ are common in adenine and hypoxanthine derivatives having substitution at their N9 position[24,25] (however absent in adenine and hypoxanthine). Therefore the aim of the experiments at basic medium (pH 12) was to look at similar transformation reaction of the N-protonated to C-protonated species. However, the spectral similarities as well as the oxidation reaction with MV^{2+} clearly rule out this kind of transformation reactions, but fully support the existence of a nitrogen protonated carbon-centered radical even at higher pH. The absence of such a transformation in the case of adenine and hypoxanthine is explained based on the assumption that the electron density at C2 and C8 positions (these are the two potential sites of carbon protonation) is low because of the deprotonation at higher pH and hence results a slower rate of protonation at these sites.[24,25] Such an explanation is equally valid in the case of 8AA, where the only one probable carbon site available for protonation might have low electron density because of the deprotonation at N9 position as 8AA has a pK_a value at around 11.5.



Scheme 4. Proposed mechanism of the reaction of e^-_{aq} with 8-azaadenine

The most probable sites of hydrogen attack in adenine systems are C2 and C8 positions in solid state.[28] In the aqueous phase, similar sites were reported in a number of structural analogs of adenine.[24,29] As the yield of H-atoms in neutral pH is only about 0.6×10^{-7} mol J^{-1}, it is convenient to perform the investigation of this reaction at acidic medium where the e^-_{aq} is fully converted to H•.[6] Therefore, the reaction mechanism may not necessarily be the same as that at neutral pH. On the other hand, in the case of adenine as well as of hypoxanthine the H• undergoes addition at C2 and C8 positions even at strongly acidic medium where these two are in their protonated forms.[24,25,30] In the present case, however, the most probable site of attack is proposed as the C2 and hence a nitrogen-centered radical of the type 8AA-C2(H)N(3)• is expected. The observed spectrum having an absorption maximum at 330 nm is therefore attributed to the formation of this radical.

CONCLUSION

The free radical chemistry of 8AA is important because of its relevance in the therapeutic applications. The reaction of hydroxyl radicals demonstrates an entirely different reaction mechanism compared to that of adenine. 8AA-4OH• at near neutral pH is quite stable in the pulse radiolysis scale whereas A-4OH• from adenine undergoes a fast water elimination reaction. While the 8AA-4OH• at higher pH undergoes a dehydration reaction leading to the formation of a N-centered radical, 8AA-N(9)•, there is an OH^- induced inhibition of OH^- elimination of the A-4-OH• from adenine, and hence it undergoes only a deprotonation reaction (i.e., A-4-$O^{\bullet -}$). The redox nature of the intermediate radicals is also different in both the cases. The formation of a substituted hydroxyl radical adduct at the C8 position of adenine which is considered as a biologically relevant reaction, is completely absent in the present case. This leads to the impression that the ring opening reaction, a major reaction in the case of adenine, is not possible with 8AA. The reactions of other radicals such as $O^{\bullet -}$, $SO_4^{\bullet -}$, e^-_{aq}, and H• proceed in a more or less similar way like in the case of adenine. On the other hand, the similarity of the radical 8AA-N(9)•, formed from the reaction of •OH at basic pH, of $O^{\bullet -}$ at pH $\approx$ 14 and of $SO_4^{\bullet -}$ at basic pH is an interesting observation.

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